UDC 547.28+547.295+547.271

OLIVOMYCIN AND RELATED ANTIBIOTICS

XIV. The Structure of Olivin*

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Khimiya Prirodnykh Soedinenii, Vol. 5, No. 4, pp. 304-316, 1969

Olivin is formed as the main product in the hydrolysis and alcoholysis of all olivomycin antibiotics [3, 4]. It is the characteristic aglycone of the olivomycins, determining their difference from the other antibiotics of this group, which are based on a higher homolog of olivin, chromomycin [5]. As a result of the investigations described below, we have established that olivin has the structure I.

Because of the tendency of olivin to undergo intermolecular association, the measurement of its molecular weight in solutions leads to different values according to the concentrations of the solutions. Extrapolation of the figures obtained to infinite dilution gave 400 ± 12 as the most probable value of the molecular weight. In combination with the results of elementary analysis and a determination of the number of protons from the NMR spectra, this led to the empirical formula for olivin $C_{20}H_{22}O_9$, which was confirmed by mass spectrometry. The results of a functional analysis and the spectral characteristics and chemical properties of olivin enabled further details of this formula to be given. It was found that olivin contains six active hydrogen atoms and readily forms a hexaacetate (cf. [4]) and, consequently, contains six hydroxyl groups. In the IR spectra of hexaacetylolivin the bands of the carbonyl absorption are located over a wide range of frequencies (1730–1780 cm^{-1}) and in the NMR spectrum of this acetate (Fig. 1) the three-proton singlets of the acetoxy groups have substantially differing chemical shifts (δ 2.0–2.5); this permits the assumption that the hydroxyls of the olivin include both alcoholic and phenolic or enolic forms.

In the same NMR spectrum in the 5.5 ppm region that is characteristic for the methine grouping of >CHOAc there is a three-proton multiplet which is absent from nonacylated olivin derivatives; this shows that three of the six hydroxyls are secondary alcoholic hydroxyls. The action of diazomethane on olivin forms a dimethyl ether (cf. [4]), the NMR spectrum of which has the signal of a hydrogen-bonded phenolic hydroxyl (one-proton peak at 9.64 ppm) which does not undergo methylation under these conditions. The product of the complete acetylation of dimethylolivin contains four acetoxy groups of which one (δ 2.30) is attached to an aromatic nucleus and the three others to methine groups, as is shown by the similarity of the NMR spectra of hexaacetylolivin and tetraacetyldimethylolivin in the 5.5 ppm region. It follows from this that as well as three secondary alcoholic groups olivin contains three phenolic (or enolic) hydroxyls. In addition, on the basis of the spectral characteristics it is possible to deduce the presence in olivin of a methoxyl on a saturated carbon atom (three-proton singlet at 3.4 ppm) a C-methyl in a $CH_2CH(-O)$ grouping (three-proton doublet at 1.3 ppm with J = 6 Hz), three aromatic protons with signals in the 7 ppm region and two carbonyls—one unconjugated (ν 1722 cm^{-1}) and one conjugated chelate carbonyl (strong band at 1640 cm^{1}; capacity for the formation of metal complexes with a bathochromic shift of the UV absorption). Thus, the empirical formula of olivin can be expanded in the following way:

$$C_{15}H_6\left\{\begin{array}{l}CO_{\text{nonconj}},\ CO_{\text{chelate}},\ 3\,OH_{\text{sec. alc}},\quad 3\,OH_{\text{(ph)enolic}},\\ OMe_{\text{alip}},\quad (O)CHMe,\ 3\,H_{\text{arom}},\end{array}\right.$$

As can be seen from this formula, the molecule of olivin contains ten increments of double bonds, two of which are due to carbonyl groups. Since no olefinic bonds can be detected in olivin either spectrally or chemically, it is not difficult to come to the conclusion that the remaining eight increments correspond to a cyclic system, the most probable being the linkage of one saturated and two aromatic rings, since the substance possesses a strong chromophore with a long-wave absorption maximum at about 405 mμ. Olivin specifically strongly increases the acidity of solutions of boric acid (ΔpH 2.5), which shows the presence in its molecule of a 1,8-dihydroxynaphthalene grouping (cf. [6]). However, it has a smaller tendency to oxidation than ordinary peri-dihydroxynaphthalenes and it is therefore natural to assume that this grouping is stabilized by a chelate bond of one of the perihydroxyls with a conjugated carbonyl group. In fact, hydrogenolysis of the conjugated carbonyl in olivin is difficult, which is characteristic for ortho-ketophenols [7] and it is

*For part XIII, see [1], and for a preliminary communication, see [2]. The subject matter of the present paper was presented in part at the International Congress on Antibiotics (Prague, 1964), and at the IX-th Mendeleev Conference on General and Applied Chemistry (Kiev, 1965).

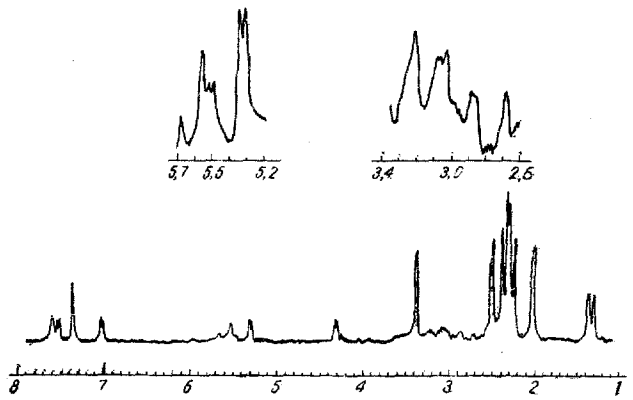
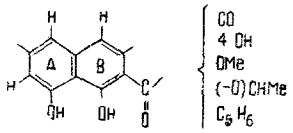


Fig. 1. NMR spectrum of hexaacetylolivin (II) (100 MHz).

accompanied by a disappearance of the strong band at 1640 cm^{-1} and also by a marked hypsochromic shift in the UV absorption; in fact, the deoxoolivin formed on hydrogenolysis is very sensitive to oxidation, as was to be expected in view of the presence in it of an isolated peri-dihydroxynaphthalene grouping. The location of the other substituents in the naphthalene nucleus of olivin follows from the NMR spectra which shows that of the three aromatic protons present in the molecule two are in the meta position with respect to one another (AB system with $J = 2.5$ Hz), and the third is in the peri position with respect to one of them (broadened singlet at 7.5 ppm). These facts permit olivin to be ascribed the following partial formula:



Under the action of acetone in the presence of copper sulfate, olivin gives an isopropylidene derivative with no change in its UV spectrum, which shows the presence of a 1,2- or 1,3-glycol grouping in the nonchromophoric part of its molecule. This isopropylidene derivative was converted by exhaustive acetylation followed by mild hydrolysis of the ketal group in olivin tetraacetate.* The latter is readily oxidized by periodate with the consumption of 2 moles of oxidizing agent and the formation of 1 mole each of acetaldehyde, formic acid, and the tetraacetyl derivative of an acid which we have called olivinic acid;

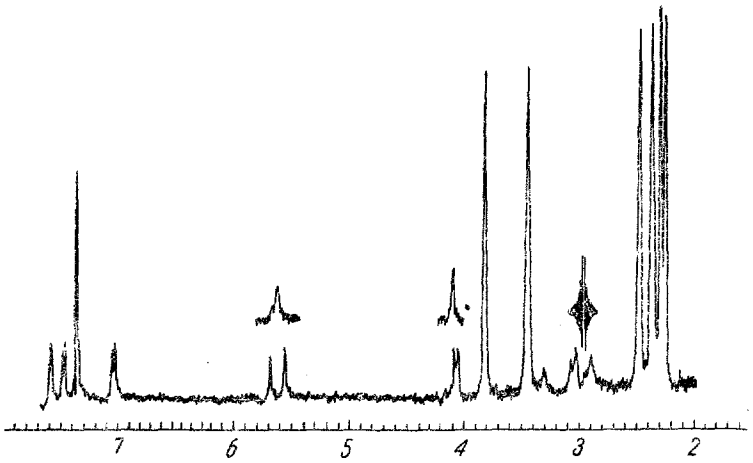


Fig. 2. NMR spectrum of methyl tetraacetylolivinate (XI) (100 MHz).

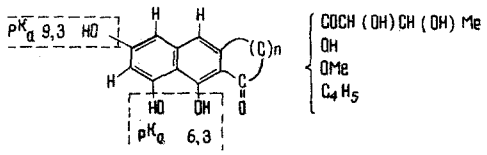
the oxidation of tetraacetylolivin with lead tetraacetate takes place similarly. These results show that olivin and tetraacetylolivin contain a COCH(OH)CH(OH)Me grouping from the carbonyl of which the carboxyl group of the tetraacetylolivinic acid is formed. **

*B. A. Klyashchitskii took part in this work.

** The alternative structure C(OH)(CHO)CH(OH)Me contradicts a number of facts, in particular the fact that all the alcoholic hydroxyls of olivin are secondary.

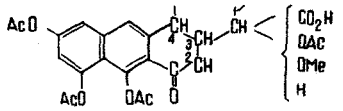
Thus, the positions of 16 out of the 20 carbon atoms of olivin have been determined. There remain only four C-atoms, which are clearly insufficient to form a bridge between rings A and B. Consequently, it is obvious that the third saturated, ring includes the chelate carbonyl and is condensed with ring B, i.e., olivin contains a linearly-fused tricyclic system. The IR frequency of the conjugated carbonyl in those derivatives of olivin in which it is not in the chelate form (1690–1705 cm^{-1}) shows that this ring is not less than six-membered.

Olivin is a weak dibasic acid with pK_a 6.3 and 9.3 [2]. The first of its acidity constants is due to a ketodiphenolic, i.e., a 2-oxo-1,8-dihydroxynaphthalene grouping. The second dissociation constant, judging from its magnitude, is connected with the presence of another phenolic hydroxyl in the naphthalene nucleus. Since, however, the molecule is linearly fused, and aromatic protons are present in the meta-peri positions, this phenolic hydroxyl can be located only in ring A, which leads to the following partial structure of olivin:



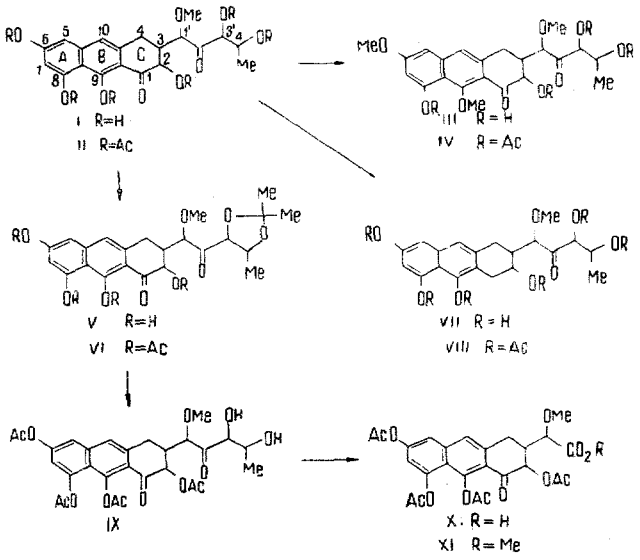
Decisive information on the structure of olivin was obtained from the NMR spectrum of the methyl ester of tetraacetylolivinic acid. In this spectrum (Fig. 2), the three peaks in the 7 ppm region correspond to three aromatic protons, while the two doublets (6.96 and 7.38 with J = 2.5 Hz) correspond to meta-hydrogens and the singlets at 7.45 ppm to peri-hydrogen. Of the four three-proton singlets between 2.2 and 2.5 ppm, three (2.30, 2.37, and 2.48) are due to aromatic acetoxyl groups and the fourth (2.26) shows the presence of an acetylated alcoholic hydroxyl. The peak at 3.79 ppm, which is absent from the spectrum of the free acid, corresponds to the methyl of the methoxycarbonyl group, and the peak at 3.42 to a methoxyl on a saturated carbon atom. The remaining five protons of the molecule are represented by two isolated one-proton doublets (5.56 and 4.07 ppm) and by a complex multiplet (in the 3 ppm region) including the signals of three protons of which one is present in a methine group and the other two in a methylene group, as can be seen from the ratio of the number of carbon and hydrogen atoms in the molecule. As the double NMR resonance spectrum shows, the appearance of a doublet at 4.07 and 5.56 ppm is due to the interaction of the corresponding H atoms with one and the same proton, the center of the signal of which is located at 2.93 ppm. Since both these H atoms appear in the form of doublets and, consequently, do not participate in other interactions characterized by an appreciable spin-spin splitting value, the multiplet in the 3 ppm region must be connected with the interaction of a methylene group with the same proton at 2.93 ppm (Fig. 2).

Thus, this proton is vicinal to two methyls and one methylene group, in other words the molecule contains the branched grouping CH–CH(CH)–CH_2. This grouping contains two of the hitherto unassigned C- and H-atoms of olivin (C_4C_5 fragment) and its structure unambiguously shows that the third saturated ring is a six-membered ring and contains a side chain in position 3. In relation to tetraacetylolivinic acid, this leaves undetermined only the positions of three functional groups and one hydrogen atom.

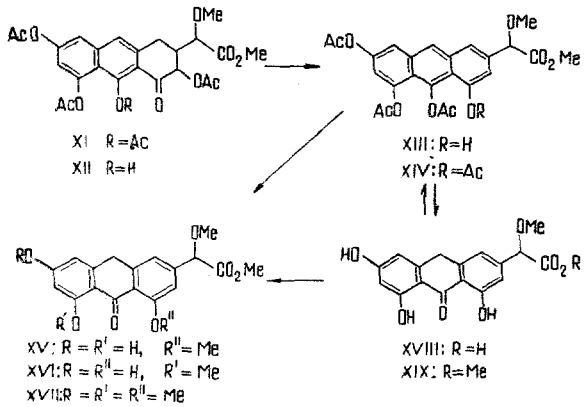


At the same time, it is obvious that the carboxyl cannot be in position 2, since tetraacetylolivinic acid is thermally stable and does not exhibit the tendency to decarboxylation which is characteristic for β-ketoacids. The methoxyl and acetoxyl (and in olivin the free hydroxyl) groups cannot be present either in the α-position to the aromatic nucleus (i.e., at C_4) or on the same carbon atom (i.e., in the form of an acylal grouping at C_1') since both these groups are stable to hydrogenolysis and acid hydrolysis. Consequently, the carboxyl is located at C_4 or C_1' and of the two OR groups one is present in position 2 and the other in position 1'. In the NMR spectrum of methyl tetraacetylolivinate (Fig. 2) the signal of the >CHOAc methine forms a doublet at 5.56 ppm with J = 12 Hz. Such a large value of the spin-spin coupling constant shows that this methine proton is rigidly fixed in the antiplanar position with respect to the neighboring H atom which occupies the axial position in ring C. Consequently, the acetoxyl group is located at C_2 and the methoxyl group in the side chain at C_1. Judging from the chemical shift of the CH_2 group (δ about 3 ppm), the methylene is more probably attached to the aromatic ring than to the methoxy group, i.e., the last unidentified proton is present in position 4. The structure following from this with a carboxyl and a methoxyl at C_1' is confirmed by the mass spectrum of methyl tetraacetylolivinate in which there are intense peaks of fragments formed as a result of the splitting off of the side chain

$CH(OMe)CH_2Me$ (103 Me) after the elimination of ketene and acetic acid: m/e 385 (M − 42 − 103), 343 (M − 42 × 2 − 103), 301 (M − 42 × 3 − 103), 283 (M − 42 × 2 − 60 − 103), 241 (M − 42 × 3 − 60 − 103). Thus, tetraacetylolivinic acid possesses the structure **X**, which leads to formula I for olivin. Consequently, the reactions of olivin described above can be represented by the following scheme:*



To confirm the proposed formulas we carried out the aromatization of the tricyclic system upon which olivin and its derivatives are based. For this purpose we subjected methyl tetraacetylolivinate (**XI**) to low-temperature vacuum pyrolysis. It was assumed, that as a result of a quasi-cyclic cis-elimination of acetoxyl and a hydrogen atom from positions 2 and 3 with subsequent γ-enolization of the enol so produced, a substituted 1-anthranol (**XIII**) would be formed. In actual fact, however, it was found that the pyrolysis product possessed the electronic spectrum characteristic for anthracenes but did not contain a free hydroxyl group and was not a tri- or tetraacetate. It had a molecular weight of 512, i.e., only 18 less than the starting substance, from which it follows that stoichiometrically the reaction consists of a dehydration and is accompanied by the intermolecular migration of an acetyl group. The donor of this group is the initial tetraacetate (XI) which, as a result of alternative degradation, is converted into the 9-hydroxy compound (XII). The position of the free hydroxyl in this follows from the fact that the IR spectrum has the band of a chelate oxo group (Scheme). Consequently, the hydrolysis product, which we have called methyl tetraacetylanhydroolivinate, was obtained in higher yield by heating compound XI with acetic anhydride, i.e., under conditions favoring the acetylation of the intermediate phenol **XIII**.



*The conclusion of the 6, 9-position of the aromatic methoxy groups in the dimethyl ether of olivin (**III**) is based on the fact that in this compound the conjugated carbonyl is nonchelate (ν 1675 cm^{-1}) and the phenolic hydroxyl forms an intramolecular hydrogen bond (δ 9.64 ppm).

The structure of the tetraacetylanhydroolivinic acid ester (XIV) follows from the information given above and also from its NMR spectrum (Fig. 3) which shows the presence of four acetoxyls (three-proton singlets at 2.33, 2.37, 2.42, and 2.53 ppm), two methoxyls (three-proton singlets at 3.44 and 3.71 ppm), an isolated methine (one-proton singlet at 5.01 ppm), and five aromatic protons not one of which is present in the ortho position to another (one-proton peaks at 7.06, 7.67, 7.72, 8.12, and 8.36 ppm with J not greater than 3 Hz).

On acid hydrolysis, the ester XIV gives unsubstituted anhydroolivinic acid which, as can be seen from the NMR, UV, and IR spectra of the acid itself and its derivatives, exists in the anthrone form (XVIII). The acetylation of the methyl ester of this acid (XIX) is accompanied by retroisomerization into an anthranol and leads to the initial tetraacetoxyanthracene (XIV). On alcoholysis with methanolic HCl solution the latter underwent deacetylation and monomethylation with the formation of the methyl ether XV or XVI retaining the nonchelate phenolic hydroxyl (ν_{OH} 3430 cm^{-1}). Under the action of an excess of diazomethane, this compound was converted into the pentamethyl derivative XVII, which was also obtained by the exhaustive methylation of anhydroolivinic acid (XVIII).

The reactions described show the linear linkage of the three rings in the molecule of olivin, while the nature of the spin-spin coupling of the aromatic protons (absence of ortho hydrogen atoms) confirms the location of the substituents in the tricyclic system—in particular, the position of the side chain. The absence of an appreciable change in the chemical shift of the two methoxyls on aromatization confirms that the methoxy and methoxycarbonyl groups are present in the side chain, and the observed paramagnetic shift of the peak of the methine of the side chain ($\Delta\delta$ 0.94 ppm) corresponds to the expected value for the transition $AlkCH(OMe)CO_2Me \rightarrow ArCH(OMe)CO_2Me$.

Experimental

The substances were isolated by thin-layer chromatography in a nonfixed layer of "vodnaya kremnevaya kislota" ["aqueous silicic acid"] silica gel (activity grade III–IV, see [8]); the R_f values relate to the system in which the separation was carried out. Unless stated otherwise, the molecular weights were determined by mass spectrometry, the IR spectra were recorded in a paraffin oil mull, the UV spectra in 96% ethanol, and the NMR spectra in $CDCl_3$ at 60 MHz with tetramethylsilane as internal standard (the chemical shifts are expressed in ppm on the δ scale; the following abbreviations are used: s) singlet; d) doublet; t) triplet, m) multiplet. * The identity of the substances obtained by different methods was shown by comparing their mp's, their $[\alpha]_D$ and R_f values and their IR and UV spectra and also, in a number of cases their NMR spectra.

Olivin (I). This was obtained by the acid degradation of olivomycins A, B, C and D [3]. Mp 189–191° C (from acetonitrile); $[\alpha]_D^{22}$ +60.5° (c 0.5; ethanol) R_f 0.59 [in the benzene–acetone (3 : 2)] system; λ_{max}, cm^{-1}: 230, 277, 355 (shoulder), 408 mμ (lg ε 4.27, 4.56, 3.64, 3.59, 4.03; $\lambda_{max}^{0.01}$ N. NaOEt in EtOH 288.314, 412 mμ (lg ε 4.53, 4.03, 4.21); $\lambda_{max}^{0.01}$ M. $AlCl_3$ in EtOH 233, 282, 327, 343, 426 mμ (lg ε 4.29, 4.47, 3.83, 3.85, 3.85, 4.01); $\lambda_{max}^{0.01}$ M. $Na_2B_4O_7$ in EtOH 227, 277, 325, 408 mμ (lg ε 4.27, 4.59, 3.63, 4.08; $\lambda_{max}^{0.01}$ M. AcONa in EtOH 223, 320, 410 mμ (lg ε 4.35, 4.61, 3.75, 4.13); ν_{max} 1590, 1640, 1722, 3400; ν_{max}^{THF}, cm^{-1} 1592, 1637, 1735, 3360; pK_a 6.3; 9.3 (by potentiometric titration in 10% ethanol [2]).

Found, %: C 58.8; H 5.6; CH_3O 8.0; $CH_3(C)$ 3.1; H_{act} 1.5; mol. wt. (thermoelectric method in ethyl acetate with an accuracy of ±3%) 404 (in 0.2 M. solution), 408 (0.03 M.), 414 (0.05 M.), 430 (0.08 M.), 462 (0.12 M.), 494 (0.16 M.); mol. wt. (m/e) 406. Calculated for $C_{20}H_{22}O_9$, %: C 59.1; H 5.5; 1 CH_3O 7.6; 1 $CH_3(C)$ 3.7; 6H_{act} 1.5; mol. wt. 406.

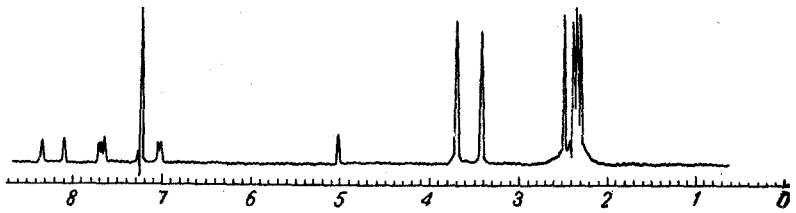


Fig. 3. NMR spectrum of methyl tetraacetylanhydroolivinate (XIV) (100 MHz).

*The mass spectra were taken by V. G. Zaikin and B. V. Rozynov, and the IR spectra by V. A. Krasnova.

Determination of the acidity of the borate complex of olivin: a 0.18 M solution of H_3BO_3 in 25% aqueous ethanol had pH 5.55; when olivin was dissolved in it to a concentration of 0.009 M, the pH fell to 3.05.

Hexaacetylolivin (II). A solution of 300 mg of olivin (I) in 1.5 ml of pyridine and 1.5 ml of acetic anhydride was kept at 20° C for 24 hr and was evaporated at 35° C/0.1 mm; the residue was dissolved in chloroform and the solution was washed with 1 N H_2SO_4 and with water and was dried and re-evaporated, and the residue was dissolved in 2 ml of ethyl acetate. On standing, 140 mg of substance crystallized out; from the mother liquor by crystallization followed by chromatography in the benzene–acetone (5 : 1) system was obtained and additional amount of product. The total yield of hexaacetylolivin (II) was 245 mg (50%); R_f 0.58; mp 200–202° C (from a mixture of chloroform and ethyl acetate); $[\alpha]_D^{23}$ −7° (c 0.5; chloroform); λ_{max}, mμ: 252 shoulder, 258, 303, 312 (shoulder), 359 (lg ε 4.70, 4.80, 3.88, 3.80, 3.58); ν_{max}, cm^{-1}: 1570, 1631, 1709, 1739, 1768; ν_{max}^{THF} 1633, 1706, 1746, 1755, 1781; δ (100 MH_Z) 1.34 (3H, d, J 6, $3H_{5'}$), 2.02 (3H, s, AcO), 2.24 (3H, s, AcO), 2.30 (3H, s, AcO), 2.32 (3H, s, AcO), 2.38 (3H, s, AcO), 2.50 (3H, s, AcO), 2.8–3.1 (3H, m, $H_3 + 2H_4$), 3.37 (3H, s, MeO), 4.29 (1H, d, J 1, $H_{1'}$), 5.28 (1H, d, J 2.5, $H_{3'}$), 5.4–5.7 (2H, m, $H_2 + H_{4'}$), 6.98 (1H, d, J 2.5, H_7), 7.46 (1H, d, J 2.5, H_5) 7.52 (1H, s, H_{10}).

Found, %: C 58.3; H 5.2; $CH_3CO(O)$ 40.3; mol. wt 658. Calculated for $C_{32}H_{34}O_{15}$, %: C 58.3; H 5.2; 6 $CH_3CO(O)$ 38.3; mol wt. 658.

6, 9-Di-O-methylolivin (III). To a solution of 500 mg of olivin (I) in 20 ml of tetrahydrofuran at 5° C was added 14 ml of a 0.6 M ethereal solution of diazomethane. The mixture was kept at the same temperature for 10 hr and was then evaporated, and the residue was chromatographed in the benzene–acetone (5 : 1) system. After crystallization from isopropanol, the yield of dimethylolivin (III) was 300 mg (56%); R_f 0.34; mp 119–126° C; $[\alpha]_D^{20}$ +95° (c 1; chloroform). λ_{max}, mμ: 225, 275, 325, 340, 379 (lg ε 4.38, 4.65, 3.68, 3.68, 3.89; ν_{max}, cm^{-1} 1563, 1643, 1675, 1722, 3300, 3420; δ 1.28 (3H, d, J 7, $3H_{5'}$), 2.6–3.0 (3H, m, $H_3 + 2H_4$), 3.50 (3H, s, alip. MeO), 3.73 (3H, s, arom. MeO), 3.90 (3H, s, arom., MeO), 4.2–4.5 (3H, m), 4.69 (1H, d, J 2.5), 6.34 (2H, s, $H_6 + H_8$), 7.09 (1H, s, H_{10}), 9.64 (1H, s, 8-OH).

Found, %: C 60.5 H 6.5; CH_3O 21.5; mol. wt. 434. Calculated for $C_{22}H_{26}O_9$, %: C 60.8; H 6.0; 3 CH_3O 21.4; mol. wt. 434.

Dimethylolivin (III) was acetylated in the same way as olivin, and after chromatography in the benzene–acetone (5 : 1) system the substance isolated was reprecipitated with petroleum ether from ethereal solution. The yield of tetra-acetyl methylolivin (IV) was 64%; R_f 0.72; λ_{max}, mμ; 225, 268, 328, 354 (lg ε 4.24; 4.60, 3.88, 3.65); ν_{max}, cm^{-1} cm^{-1}: 1570, 1630, 1700, 1743; $\nu_{max}^{CHCl_3}$ 1570 1627, 1697, 1745 cm^{-1}; δ 1.30 (3H, d, J 6, $3H_{5'}$), 2.00 (3H, s, ACO); 2.20 (3H, s, AcO), 2.25 (3H, s, AcO), 2.30 (3H, s, AcO), 2.8–3.2 (3H, m, $H_2 + 2H_4$), 3.35 (3H, s, alip. MeO), 3.80 (6H, s, 2 arom. MeO), 4.20 (1H, s, $H_{1'}$), 5.3–5.7 (3H, m, $H_2 + H_{3'} + H_{4'}$), 6.65 (2H, m, $H_6 + H_8$), 7.15 (1H, s, H_{10}).

Hexaacetyl-1-deoxoolivin (VIII). A solution of 203 mg of olivin (I) in 20 ml of ethanol was hydrogenated in the presence of a platinum catalyst (from 100 mg of PtO_2) until 26.2 ml of H_2 had been adsorbed. The solution was filtered and evaporated, and the residue was evaporated with chloroform and the mixutre was again evaporated. The resulting 1-deoxoolivin (VII) [λ_{max} mμ 220, 237, 275, 340 (lg ε 4.38, 4.26, 3.87, 3.95); ν_{max} cm^{-1}: 1612, 1722, 3350; under the conditions described above for olivin, $\Delta pH_{H_3BO_3}$ 2.5] was acetylated with 1 ml of acetic anhydride in 1 ml of pyridine as in the production of (II). The yield of the hexaacetate VIII was 115 mg (37%); R_f 0.59 in the benzene–acetone (5 : 1) system; mp 192–193° C from ethyl acetate); $[\alpha]_D^{22}$ −25° (c, chloroform); λ_{max}, mμ: 234, 290 (log ε 4.99, 3.83); ν_{max}, cm^{-1}: 1573, 1615, 1640, 1737, 1762; δ 1.25 (3H, d, J 6, $3H_{5'}$), 1.97 (3H, s, AcO), 2.10 (3H, s, AcO), 2.22 (3H, s, AcO), 2.27 (3H, s, AcO), 2.35 (3H, s, AcO), 2.41 (3H, s, AcO), 3.33 (3H, s, MeO), 4.26 (1H, d, J 2.5, $H_{1'}$), 5.1–5.8 (3H, m, $H_2 + H_{3'} + H_{4'}$), 6.90 (1H, d, J 2.5, H_7), 7.47 (2H, $H_5 + H_{10}$).

Found, %: C 59.2; H 5.8; mol. wt. 644. Calculated for $C_{32}H_{36}O_{14}$. %: C 59.7; H 5.6; mol. wt. 644.

3', 4'-O-Isopropylideneolivin (V). A mixture of 203 mg of olivin (I) and 200 mg of anhydrous $CuSO_4$ in 12 ml of absolute acetone was stirred at 25–30° C for 2 days after which the solution was filtered and evaporated and the residue was chromatographed in the benzene–acetone (5 : 2) system. From the zone with R_f 0.50–0.60 was isolated 197 mg (86%) of isopropylideneolivin (V); $[\alpha]_D^{22}$ −31° (c 1; ethanol; λ_{max}, mμ: 231, 276, 326, 406 (lg ε 4.25, 4.58, 3.60, 4.09); ν_{max}, cm^{-1} 1588, 1640, 1724, 3355.

Found, %: C 61.3; H 6.1. Calculated for $C_{23}H_{26}O_9$. %: C 61.4; H 5.8.

2, 6, 8, 9-Tetra-O-acetyl-3', 4'-O-isopropylideneolivin (VI). This was obtained by the acetylation of isopropylideneolivin (V) under the conditions described above for hexaacetylolivin (III). Yield 92%; R_f 0.62 in the benzene–acetone (5 : 1) system; $[\alpha]_D^{22}$ −42° (c 1, chloroform); λ_{max} mμ: 252 (shoulder), 259, 305, 360 (lg ε 4.69, 4.79, 3.81, 3.44); ν_{max}, cm^{-1} 1570, 1630, 1703, 1726, 1777; δ (100 MHz) 1.36 and 1.42 (6H, 2s, Me_2C), 2.27 (3H, s, ACO), 2.31 (3H, s, AcO), 2.36 (3H, s, AcO), 2.47 (3H, s, AcO), 3.39 (3H, s, MeO), 4.12 (2H), 4.37 (1H, d, J 2.5, $H_{1'}$). 5.56 (1H, d, J 12, H_2), 6.98 (1H, d, J 2.5, H_7), 7.44 (1H, d, J 2.5, H_5), 7.52 (1H, s, H_{10}).

Found, %: C 60.4; H 5.7. Calculated for $C_{31}H_{34}O_{13}$, %: C 60.6; H 5.5.

2,6,8,9-Tetra-O-acetylolivin (IX). A solution of 200 mg of the isopropylidene derivative VI in 10 ml of 50% acetic acid was heated at 75° C for 50 min and was then evaporated, after which the residue was dissolved in 20 ml of chloroform and the solution was washed with water, dried, and evaporated again. By chromatography in the benzene–acetone (3:1) system, the residue yielded 144 mg (77%) of tetraacetylolivin (IX); R_f 0.53; $[\alpha]_D^{22}$ − 7° (c 1, chloroform); λ_{max} mμ: 252 (shoulder), 259, 305, 314, 357 (lg ε 4.69, 4.79, 3.81, 3.44); ν_{max}, cm^{-1}: 1570, 1630, 1703, 1730 (shoulder), 1772, 3455; δ 1.28 (3H, d, J 6, $3H_5$'), 2.24 (3H, s, AcO), 2.31 (3H, s, AcO), 2.38 (3H, c, AcO), 2.47 (3H, s, AcO), 3.37 (3H, s, MeO), 4.38 (1H, s, H_1'), 6.96 (1H, d, J 2.5, H_7), 7.38 (1H, d, J 2.5, H_5), 7.49 (1H, s, H_{10}).

Found, %: C 58.8; H 5.4. Calculated for $C_{28}H_{30}O_{13}$, %: C 58.8; H 5.2.

Tetraacetylolivinic acid (X). A) A solution of 200 mg of tetraacetylolivin (IX) in 7 ml of methanol was mixed with 30 ml of a 0.03 M aqueous solution of $NaIO_4$ and left in the dark at 20° C for 1 hr. The precipitate was filtered off (104 mg). Ethyl acetate extracted another 78 mg of substance from the filtrate. After crystallization from ethanol, the yield of tetraacetylolivinic acid (X) in the form of dihydrate was 162 mg (84%); mp 126–128° C; $[\alpha]_D^{22}$ −45° (c 1; chloroform); R_f 0.57 in the benzene–acetone (5:2) system; λ_{max}, mμ: 250 (shoulder), 258, 305, 315, 355, lg ε 4.75, 4.84, 3.90 3.83, 3.54); ν_{max}, cm^{-1}: 1570, 1630, 1703, 1764, 3420.

Found, %: C 54.3; H 5.2. Calculated for $C_{25}H_{24}O_{12} \cdot 2H_2O$, %: C 54.4; H 5.1.

B) To a solution of 200 mg of tetraacetylolivin (IX) in 25 ml of acetic acid was added 1.55 g of $Pb(OAc)_3$. The mixture was stirred at 20° C for 48 hr, diluted with 25 ml of water, and extracted with benzene, after which it was acidified to pH 3 and was extracted with benzene again. The combined extracts were washed with water, dried, and evaporated. The residue was recrystallized from ethanol; by chromatography in the benzene–acetone (5:2) system, the mother solution yielded an additional amount of substance. The total yield of the acid X was 133 mg (69%); mp 124° C; $[\alpha]_D^{22}$ −45° (c 1, chloroform).

Kinetics and stoichiometry of the periodate oxidation of tetraacetylolivin (IX). A solution of 10 mg of tetraacetylolivin (IX) in 5 ml of methanol cooled to −15° C was treated with 10 ml of a 0.032 M solution of $NaIO_4$ and 35 ml of ice water, and the mixture was left in the dark at 0° C. An aliquot of the solution was made alkaline with solid $NaHCO_3$, and then an excess of KI was added and the iodine liberated was triturated with 0.02 N Na_3AsO_3. The consumption of oxidizing agent (in moles/mole) was: after 15 min, 1.33; 30 min, 1.45; 1 hr, 1.94; 1.5 hr, 2.06; 3 hr, 2.06.

A solution of 50 mg of tetraacetylolivin (IX) in 1 ml of methanol at 10° C was treated with 10 ml of a 0.032 M solution of $NaIO_4$ and the mixture was left in the dark at 10° C for 2 hr. The tetraacetylolivinic acid (X) formed was filtered off, the filtrate was made alkaline with KOH (to phenolphthalein) and the acetaldehyde together with the methanol was distilled off into 10 ml of a 0.25% solution of 2,4-dinitrophenylhydrazine in 10% H_2SO_4. This gave 17.2 mg (88%) of acetaldehyde dinitrophenylhydrazone, mp 147° C (from ethanol), R_f 0.85 [on Al_2O_3 of activity grade II in the benzene–acetone (10:1) system]; in a blank test with an equal amount of acetaldehyde, the yield of dinitrophenylhydrazone was 90%. The solution remaining after the distillation of the acetaldehyde was brought to pH 3 with H_3PO_4 and evaporated at 100° C, and the distillate was analyzed by the calomel method for formic acid [9]. The amount of Hg_2Cl_2 found was 28.8 mg (70%); in a blank experiment the yield was 88%.

Methyl tetraacetylolivinate (XI). A solution of 100 mg of the acid X in 10 ml of tetrahydrofuran was methylated with 0.8 ml of a 0.7 M ethereal solution of diazomethane (20° C, 15 min). The yield of the methyl ester XI was 90.5 mg (86%); mp 171–172° C (from ethanol); $[\alpha]_D^{22}$ −50° (c 1; chloroform); R_f 0.87 in the benzene acetone (3:1) system; λ_{max}, mμ: 250 (shoulder), 258, 304, 315, 355 (lg ε 4.81, 4.93, 3.98, 3.91, 3.66); ν_{max}, cm^{-1} 1570, 1630, 1695, 1730, 1740, 1755, 1770; δ (100 MHz) 2.26 (3H, s, AcO), 2.30 (3H, s, AcO), 2.37 (3H, s, AcO), 2.48 (3H, s, AcO), 3.42 (3H, s, C_1', OMe), 3.79 (3H, s, CO_2Me), 4.07 (1H, d, J 2.5, H_1'), 5.56 (1H, d, J 12, H_2), 6.96 (1H, d, J 2.5, H_7), 7.38 (1H, d, J 2.5, H_5), 7.45 (1H, s, H_{10}).

Found, %: C 59.1; H 5.2; mol. wt. 530. Calculated for $C_{26}H_{26}O_{12}$. %: C 58.9; H 4.9; mol. wt. 530.

Methyl tetraacetylanhydroolivinate (XIV). A) A solution of 20 mg of the ester XI in 1.5 ml of acetic anhydride was heated in a sealed tube at 205° C for 5 hr and was then evaporated to dryness. The residue was dissolved in 10 ml of chloroform, the solution was washed with water, dried, and evaporated, and the residue was chromatographed in the benzene–acetone (8:1) system, the zones with R_f 0.30–0.38 being taken. The yield of the ester XIV was 18 mg (92%); mp 221–223° C (from ethanol); $[\alpha]_D^{20}$ +84° (c 1; chloroform); λ_{max}, mμ: 227, 254 (shoulder), 262, 355, 374, 394 (lg ε 4.14, 4.79, 5.13, 3.71, 3.71, 3.69); ν_{max}, cm^{-1} 1640, 1760; δ (100 MHz) 2.33 (3H, s, AcO), 2.37 (3H, s, AcO), 2.42 (3H, s, AcO), 2.53 (3H, s, AcO), 3.44 (3H, s, C_1'-OMe), 3.71 (3H, s, CO_2Me), 5.01 (1H, s, H_1'), $5H_{arom}$; 7.06 (d, J 2.5), 7.67 (s), 7.72 (d, J 2.5), 8.12 (s), 8.36 (s).

Found, %: C 60.6; H 4.9; mol. wt. 512. Calculated for $C_{26}H_{24}O_{11}$. %: C 60.9; H 4.7; mol. wt. 512.

B) A solution of 100 mg of the ester **XI** in 7 ml of acetic anhydride was heated at 140° C in a current of argon for 96 hr and, after cooling, it was evaporated and treated as in experiment A. The yield of the ester **XIV** was 60 mg (62%), mp 221–223° C (from ethanol).

C) The ester **XI** (59 mg) was heated at 190° C/0.1 mm for 3 hr, after which it was chromatographed in the benzene–acetone (10 : 1) system. The zone with R_f 0.30–0.38 yielded 11.5 mg (20%) of the ester **XIV**, the zone with R_f 0.39–0.45 yielded 13 mg (22%) of the initial ester XI with mp 171–172° C (from ethanol), and the zone with R_f 0.50–0.62 yielded 21 mg (37%) of 2, 6, 8-triacetylolivinate (**XII**) with $[\alpha]_D^{23}$ −11° (c 1; ethanol); λ_{max}, mμ; 225, 260 (shoulder), 268, 293 (shoulder), 304, 385 (lg ε 4.42, 4.62, 4.70, 3.80, 3.82, 3.86); ν_{max}, cm^{-1}: 1580, 1632, 1754; δ 2.30 (3H, s, AcO), 2.35 (3H, s, AcO), 2.38 (3H, s, AcO), 3.50 (3H, s, C_1-OMe), 3.89 (3H, s, CO_2Me), 4.05 (1H, d, J 2.5, H_1'); 5.76 (1H, d, J 11, H_2), 6.95 and 7.30 (3Harom), 13.87 (1H, s, 9-OH).

Found, %: C 59.4; H 5.0. Calculated for $C_{24}H_{24}O_{11}$, %: C 59.0; H 5.0.

When this substance was acetylated with acetic anhydride in pyridine (20° C, 24 hr), the tetraacetate **XI** with mp 171.5–172.5° C (from ethanol) was formed.

Anhydroolivinic acid (XVIII). A solution of 100 mg of the ester XIV in 5 ml of acetic acid and 5 ml of 5 N H_2SO_4 was heated in a current of argon at 100° C for 40 min. After cooling, the mixture was extracted with ethyl acetate, the extract was dried and evaporated, and the residue was chromatographed in the benzene–acetone (8 : 3) system, the zone with R_f 0.42–0.54 being taken. This gave 50 mg (74%) of anhydroolivinic acid (XVIII) in the form of monohydrate, mp* 190° C (decomp., from ethanol); $[\alpha]_D^{23}$ +70.5° (c 0.9; ethanol); λ_{max}, mμ: 225, 258, 308, 362, (lg ε 4.27, 3.98, 4.18, 4.01); ν_{max}, cm^{-1}: 1585, 1615, 1640, 1740, 3300, 3480; $\delta^{(CH_3)_2CO+D_2O}$ 3.58 (3H, s, arom. MeO), 4.37 (2H, s, $2H_{10}$), 5.29 (1H, s, H_1'), 6.25 (1H, s, H_7), 6.60 (1H, s, H_5), 7.60 (1H, s, H_2), 7.86 (1H, s, H_4).

Found, %: C 58.8; H 5.1; Calculated for $C_{17}H_{14}O_7 \cdot H_2O$, %: C 58.6; H 4.9.

Methyl anhydroolivinate (XIX). A solution of 30 mg of the acid XVIII in 1 ml of tetrahydrofuran was mixed at 0° C with 0.24 ml of a 0.4 M ethereal solution of diazomethane and the mixture was slowly evaporated. The residue was chromatographed in the benzene–acetone (5 : 1) system. This gave 17 mg (54%) of the monohydrate of the methyl ester XIX with R_f 0.54; mp 234–235° C (from ethanol); $[\alpha]_D^{25}$ +80° (c 1; acetone); λ_{max}, mμ: 230, 257, 273, 314, 365 (lg ε ε 4.38, 4.06, 3.95, 4.18, 4.00); ν_{max}, cm^{-1}: 1580, 1610, 1640, 1710, 1730, 3430; δ^{THF} 4.10 (2H, s, $2H_{10}$), 5.16 (1H, s, H_1'), 6.12 (1H, d, J 2.5, H_7), 6.25 (1H, d, J 2.5 H_5), 7.33 (1H, s, H_2), 7.52 (1H, s, H_4), 9.05 (1H, 6-OH), 13.12 (1H, s, 1-OH or 8-OH).

Found, %: C 59.4; H 5.3; CH_3O 15.0; mol. wt. 344. Calculated for $C_{18}H_{16}O_7 \cdot H_2O$, %: C 59.7; H 5.0; 2 CH_3O 17.0; mol. wt. (anhydrous) 344.

When this substance was acetylated with acetic anhydride in pyridine (20° C, 24 hr), the tetraacetate XIV was formed. Yield 66%; R_f 0.56 in the benzene–acetone (8 : 1) system; mp 205–207° C (from ethanol); $[\alpha]_D^{30}$ +72° (c 1; chloroform).

Methyl 1- (or 8-) -O-methylanhydroolivinate (XV) or (XVI). A solution of 20 mg of the ester (**XIV**) in 5 ml of 1.5 M methanolic HCl was boiled in a current of argon for 2 hr. The crystals that deposited on cooling were filtered off and by chromatography in the benzene–acetone (5 : 1) system the mother liquor yielded an additional amount of substance. The total yield of the ester XV or XVI was 11 mg (79%); R_f 0.74; mp 241–243° C (from ethanol); $[\alpha]_D^{24}$ +50° (c 0.25; tetrahydrofuran); λ_{max}, mμ: 223, 255, 273, 305, 365 (lg ε 4.44; 4.11; 4.05; 4.25; 4.04); ν_{max}, cm^{-1} 1580, 1610, 1640, 1730, 3430; δ^{Me_2SO} 4.20 (2H, s, $2H_{10}$), 5.23 (1H, s, H_1'), 6.33 (1H, d, J 3, H_7), 6.51 (1H, d, J 3, H_5), 7.32 (1H, s, H_2), 7.57 (1H, s, H_4), 10.34 (1H, s, 6-OH), 13.33 (1H, s, 8-OH or 1-OH).

Found, %: C 63.7; H 5.0; mol. wt. 358. Calculated for $C_{19}H_{18}O_7$. %: C 63.7; H 5.1; mol. wt. 358.

Methyl 1, 6, 8-tri-O-methylanhydroolivinate (VII). A solution of 10 mg of the trihydroxy ester XIX in 1 ml of tetrahydrofuran was methylated with 0.6 ml of a 0.55 M ethereal solution of diazomethane (20° C, 1 hr), and, after evaporation, the product was chromatographed in the benzene-acetone (5 : 1) system. The yield of the ester XVII was 9 mg (77%); R_f 0.85; mp 202–204° C (from ethanol); λ_{max}, mμ: 230, 280 (shoulder), 283, 306 (shoulder), 375 (lg ε 3.99, 4.11, 4.12, 3.80, 3.40); ν_{max}, cm^{-1}: 1603, 1632, 1674, 1732; mol. wt. 386.

The same substance was obtained by the analogous methylation of the acid XVIII and the ester XV or XVI.

*A clear transition is observed only in the method of determining the melting point in a constant-temperature bath (block) [10]; otherwise the substance gradually decomposes on heating, without melting.

Conclusions

On the basis of its chemical properties and spectral characteristics, structure I has been established for the aglycone of the olivomycin antibiotics, olivin.

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3 January 1968

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