OLIVOMYCIN AND RELATED ANTIBIOTICS

XXX. THE STRUCTURE OF CHROMOCYCLIN*

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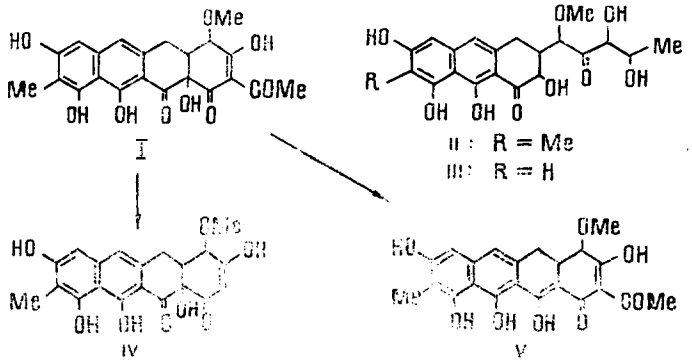
In a study of aureolic acid [2, 3] from the culture liquid of Streptomyces La-7017, we isolated, in addition to this antibiotic, another substance which possessed similar physicochemical properties but which was biologically almost inactive. Like aureolic acid, this substance is a glycoside which is readily cleaved by the action of acids. Its aglycone contains the whole chromophoric system of the initial compound and because of its structural similarity to chromomycinone (II) and tetracycline, it has been called chromocyclin and the glycoside itself chromocyclomycin [1]. As a result of the investigations described below, we have established that chromocyclin has structure (I).



The simultaneous formation of aureolic acid and chromocyclomycin by the same microorganism and the similarity of their spectra permitted the assumption, even in the early stages of the investigation, that the aglycones of these substances – chromomycinone (II) and chromocyclin – were structurally similar. In actual fact, the NMR spectra show that chromocyclin, like chromomycinone (II) has two isolated aromatic protons (singlets at 6.67 and 6.84 ppm; in chromomycinone they are at 6.61 and 6.67 ppm), a methyl group in an aromatic nucleus (2.18 ppm; in chromomycinone 2.15 ppm), and three phenolic hydroxyls (9.20, 9.75, and 15.48 ppm) corresponding to the 6-OH, 8-OH, and 9-OH hydroxyls of chromomycinone (9.52, 9.77, and 15.68 ppm). These facts permit the conclusion that chromocyclin contains the whole tricyclic moiety of the chromomycinone molecule. This conclusion agrees with the value of the second acidity constant of chromocyclin (pK_a'' 6.8), which is close to the acidity constant of the 1,8-dihydroxy-2-oxonaphthalene† grouping of chromomycinone (pK_a' 6.6) and is confirmed by the results of a comparison of the mass spectra of chromocyclin and of chromomycinone (Fig. 1). In these spectra, in the region of high mass numbers the strongest peak is that of a rearranged ion with m/e 272, and the nature of the further fragmentation is very similar in the two compounds. This ion probably possesses structure (VI) and is formed from chromomycinone by a McLafferty rearrangement and from chromocyclin by a retrodiene cleavage. The assignment of this peak is in harmony with the mass spectrum of olivin (III), in which, because of the absence of a

* For a preliminary communication see [1]; for Communication XXIX, see [2].

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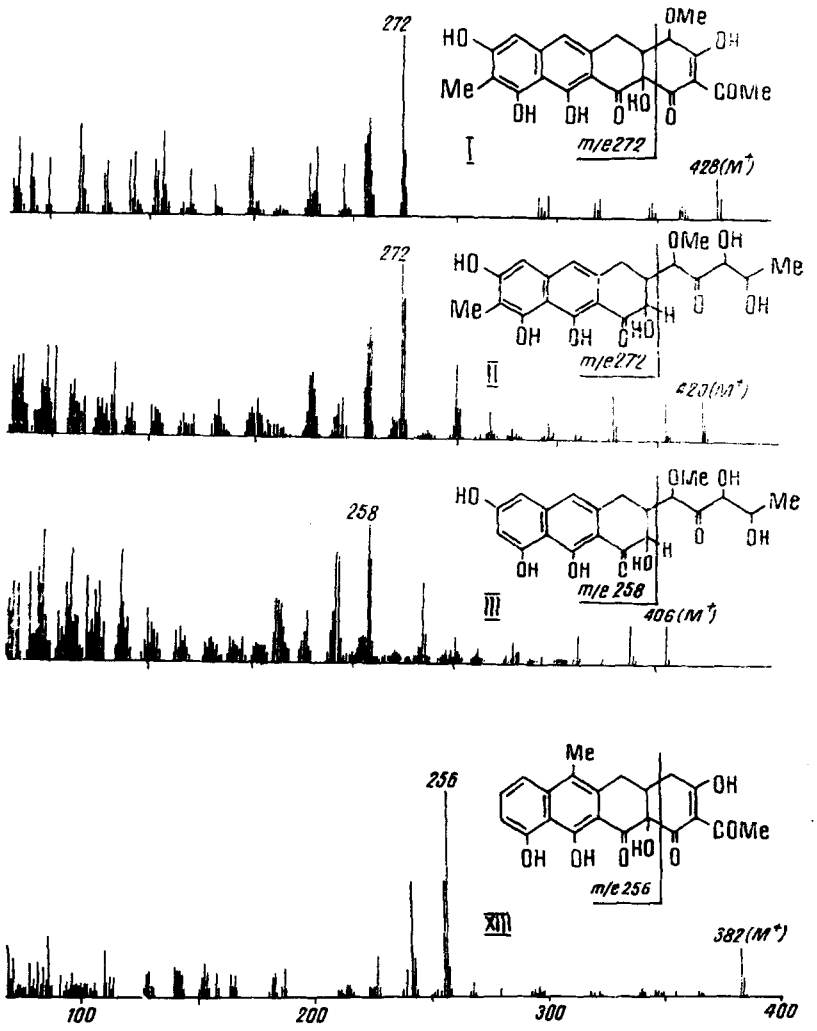
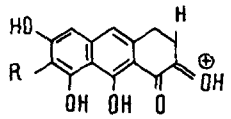


Fig. 1. Mass spectra of chromocyclin (I), chromomycinone (II), olivin (III), and acetyldecarboxamidoanhydrotetracycline (XIII).

methyl group in the tricyclic part of the molecule, the corresponding ion has m/e 258 (formula VII) and all the peaks obtained are shifted by 14 m/e to lower mass numbers.



VI: R = Me

VII: R = H

Fundamental structural information is also obtained by a comparison of the UV spectra of chromocyclin and chromomycinone (II). By deducting the absorption curve of chromomycinone (II) from the corresponding curve of chromocyclin, it was established that the spectrum of chromocyclin is the sum of the absorptions of the tetrahydroanthracene chromophore of chromomycinone and another, shorter-wave, chromophore with absorption maxima at about 250 and 300 nm, similar in its characteristics to the dimedone chromophore (Fig. 2); at the same time, the high degree of additivity shows that the two chromophores are separated and interact little with one another. The presence in chromocyclin of a cyclohexane-1,3-dione grouping is confirmed by the magnitude of its first acidity constant (pK_a' 4.8; compare dihydroresorcinol, pK_a 5.26 [4]).

In the NMR spectrum of chromocyclin, in addition to the signal of an aromatic methyl group, there is another three-proton singlet in a slightly weaker field (δ 2.51 ppm). When chromocyclin was heated with dilute sulfuric acid, a molecule of acetic acid was liberated with the formation of deacetylchromocyclin, in

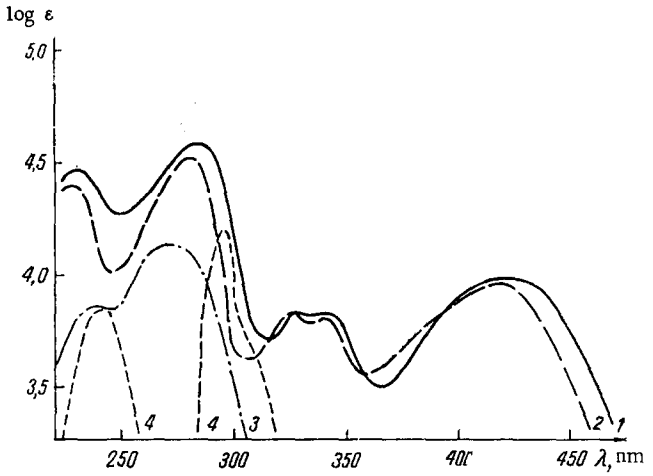


Fig. 2. UV spectra of chromocyclin (I) (1), chromomycinone (II) (2), and acetyldimedone (3), and the difference curve (I–II) (4).

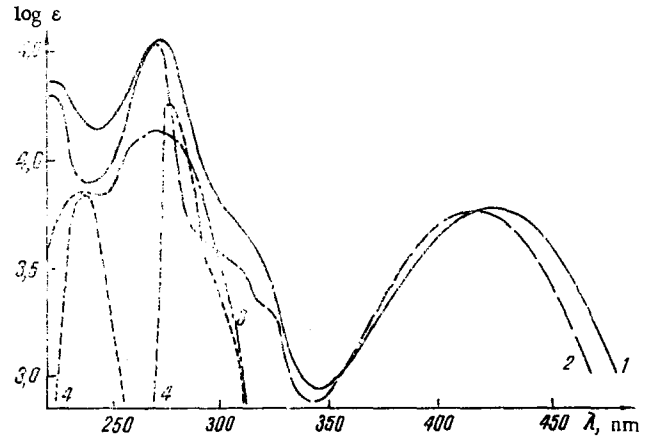
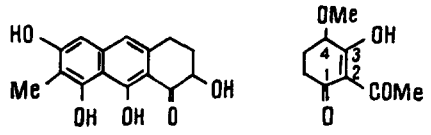


Fig. 3. UV spectra of acetyldecarboxamidoanhydrotetracycline (XIII) (1) anhydrotricyclin (XII) (2), and acetyldimedone (3), and the difference curve (XIII–XII) (4).

the NMR spectrum of which this signal had disappeared. Since chromocyclin does not contain isolated carbonyls (its IR spectrum lacks a C=O bond above 1680 cm^{-1}), the acetyl groups split out must have been attached to carbon and have been included in a conjugated system. The similarity of the fragmentation pathways of chromocyclin and chromomycinone and also the closeness of their UV spectra show that this C-acetyl group cannot be located in the aromatic part of the molecule and, consequently, must be conjugated with the carbonyls of the dihydroresorcinol grouping. Its precise position – at C_2 – follows from the NMR spectra, in which there is a one-proton signal at 18.41 ppm which is similar to the signal of the chelate proton of the tricarbonylmethane grouping of acetyldimedone (δ 18.09). Obviously, because of cross-conjugation the presence of the C-acetyl group has little effect on the short-wave band of the UV absorption of chromocyclin.

Finally, chromocyclin contains one methoxy group (determined by the Zeisel method) which is represented in the NMR spectrum by a three-proton singlet at 3.73 ppm (the corresponding signal in chromomycinone is at 3.48 ppm). The similarity of the mass spectra of chromocyclin and chromomycinone (II) shows that this group is located in the dihydroresorcinol ring in the α position to a ketonic carbonyl. Thus, the molecule of chromocyclin contains fragments (VII) and (IX). (See scheme on following page.)

The total number of carbon atoms in the fragments (VIII) and (IX) is two more than in chromocyclin, from which it follows that these two atoms are common to the two fragments, and in chromocyclin these

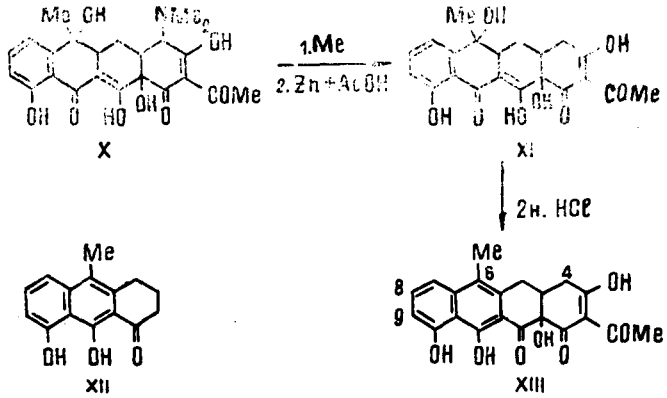


VIII IX

are connected in a condensed tetracyclic system. The reduction of chromocyclin with zinc in acetic acid leads to the hydrogenolysis of the alcoholic hydroxy group with the formation of deoxychromocyclin, which leads to a considerable bathochromic shift of the long-wave UV absorption maxima (420→476 nm). This shift shows that in deoxychromocyclin the acetyldihydroresorcinol chromophore (IX) is conjugated with the tricyclic chromophore (VIII); i.e., the oxo groups of the two chromophores are separated by one carbon atom to which a tertiary hydroxyl is attached in chromocyclin. The considerations discussed have led to formula (I) for chromocyclin and, correspondingly, to formulas (IV) and (V) for deacetyl- and deoxychromocyclins, respectively.

To confirm these formulas, from natural 2-acetyl-2-decarboxamidotetracycline (X) [5], by the method illustrated in the scheme, we obtained the model compound (XIII), which differs from chromocyclin (I) only be the absence of a methoxy group (at C_4) and one of the phenolic hydroxyls (at C_8) and also by the position of the methyl group in the aromatic nucleus (at C_6 instead of C_9).

It was found that if the UV absorption curve of the tricyclic compound (XII) [6] was subtracted from the curve of this anhydrotetracycline derivative (XIII), the resulting spectrum (Fig. 3) was close to that of acetyldimedone and scarcely differed from the difference curve obtained from the spectra of chromocyclin (I) and chromomycinone (II) (see Fig. 2). From the nature of the fragmentation on electron impact, the compounds of the anhydrotetracycline series, especially (XIII), are also similar to chromocyclin (see Fig. 1), and the splitting off of the 12a-hydroxy group from these substances causes an analogous change in the UV spectrum (see [7]). A final proof of the structure of chromocyclin by the synthesis of the product of its degradation is described in the following paper.

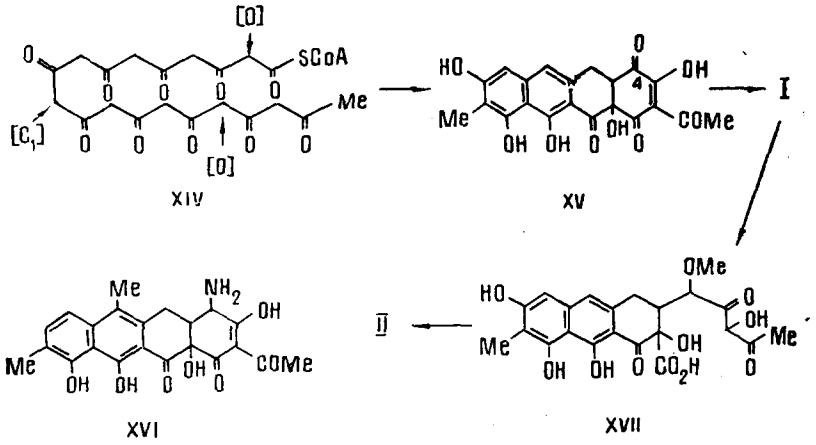


The structural similarity of chromocyclin (I) to chromomycinone (II) and the tetracyclines (for example, X) shows the probable biogenetic closeness of these substances. On the basis of modern ideas on the biogenesis of the tetracyclines [8], it may be assumed that the acetogenin (XIV), which is analogous to the hypothetical precursor of the tetracyclines, undergoes four intramolecular crotonic condensations, C_9-methylation, and C_{12a}-hydroxylation with the formation of the tetracyclic 4-ketone (XV). The reduction of this ketone and the methylation of the 4-hydroxy group so formed give chromocyclin (I), the oxidative hydrolytic cleavage of which to the oxo acid (XVII) with subsequent decarboxylation and reduction of the C-acetyl group leads to chromomycinone (II) (similar considerations are apparently valid in relation to the antibiotic chelocardin (XVI) [9]). Since both chromocyclin (I), which is, according to this scheme, an intermediate link in the biosynthesis of chromomycinone, and chromomycinone (II) itself are produced by the cell in the form of glycosides, it may be assumed that in the biogenetic chain leading to the tetracyclines there are also carbohydrate-containing compounds (see scheme on following page).

EXPERIMENTAL

Chromatography was performed in a thin nonfixed layer of silica gel of the "aqueous silicic acid" type (less than 150 mesh, activity grade III-IV). The molecular weights were determined mass-spectrometric-

ally. The IR spectra were taken of mulls in paraffin oil and the UV spectra in 96% ethanol (sh denotes a shoulder). The analyses of all the compounds corresponded to the calculated figures.



XIV XV XVI XVII

1. Chromocyclomycin. A mixture of the substances obtained from 60 liters of the culture filtrate of Streptomyces LA-7017 [3] was chromatographed in the benzene–acetone (1 : 1) system. This gave 2.0 g of aureolic acid (R_f 0.36) [3] and 2.2 g of chromocyclomycin (R_f 0.74). Chromocyclomycin, $C_{48}H_{64}O_{21}$, is a yellow crystalline substance with mp 196-198°C (from acetone): $[\alpha]_D^{27}$ −180° (c 0.3; ethanol); λ_{max} 229, 283, 322, 335, 420 nm (log ε 4.54; 4.74; 4.21; 4.13; 4.02); ν_{max} 1520, 1585, 1632, 1720, 3440 cm^{-1}; pK_a 4.6; 7.2 (in 10% ethanol).

2. Chromocyclin (I). A solution of 300 mg of chromocyclomycin in 10 ml of 0.4 N H_2SO_4 and 10 ml of methanol was heated at 80°C for 3.5 h, and after cooling it was evaporated and extracted with ethyl acetate. The residue after the evaporation of the extract was triturated with ether and the solid matter was filtered off; petroleum ether precipitated an additional amount of the substance from the mother solution (total yield 126 mg). This substance was chromatographed in the benzene–acetone (5 : 1) system. The zone with R_f 0.70-0.80 yielded 94 mg (72%) of chromocyclin $C_{22}H_{20}O_9$ (I); mp 226-228°C (from acetonitrile); $[\alpha]_D^{27}$ −435° (c 0.5; ethanol); pK_a 4.8 and 6.8 (in 10% ethanol); mol. wt. 428; λ_{max} 232, 284, 328, 420 nm (log ε 4.46; 4.67; 3.91; 4.03; ν_{max} 1540, 1575, 1640, 1660, 1690, 3420 cm^{-1}; δ $(CD_3)_2CO$ 2.18 (3H, s., C_9-Me), 2.51 (3H s., C_2-COMe), 3.73 (3H, s., C_4-OMe), 4.71 (1H, d., J 4, H_4), 6.67 (1H, s., H_7), 6.84 (1H, s., H_6), 9.20 (1H, s., O_8-H), 9.75 (1H, s., O_{10}-H), 15.48 (1H, s., O_{11}-H), 18.41(1H, s., O_3-H).

3. 2-Deacetylchromocyclin (IV). A solution of 500 mg of chromocyclin (I) in 30 ml of dioxane and 30 ml of 2 N H_2SO_4 was boiled for 30 h, and after cooling it was diluted twofold with water, and the precipitate was filtered off and washed with water. The mother solution was concentrated twofold, diluted with water, and again evaporated, whereupon acetic acid was found in the combined filtrates (by titration to phenolphthalein, 0.87 mole of AcOH/mole of chromocyclin was found; p-bromophenacyl ester: mp 85°C). The residue after evaporation was extracted with ethyl acetate and the extracted substance together with the precipitate obtained previously was chromatographed in the benzene–acetone (3 : 1) system. The zone with R_f 0.68-0.79 gave 52 mg (10%) of the initial compound (I), and the zone with R_f 0.41-0.52 gave 257 mg (57%) of 2-deacetylchromocyclin, $C_{20}H_{10}O_8$ (IV); mp 169-171°C (from a mixture of ethyl acetate and heptane); $[\alpha]_D^{20}$ −69° (c 0.15; acetone); mol. wt. 386, λ_{max} 231, 276, 326, 340, 418 nm (log ε 4.40; 4.68; 3.89; 3.90; 4.01), ν_{max} 1465, 1600, 1635, 3185, 3465 cm^{-1}.

4. 12a-Deoxychromocyclin (V). A solution of 200 mg of chromocyclin (I) in 15 ml of glacial AcOH and 600 mg of activated Zn dust was stirred at 20°C for 24 h, and then after every 4 h another 100 mg of Zn dust was added until a spot with R_f 0.81 appeared on a chromatogram in the benzene–acetone (10 : 1) system [in which chromocyclin (I) has R_f 0.63 and 12a-deoxychromocyclin (V) has R_f 0.68], which required about 60 h. The solid matter was filtered off and washed with acetic acid, ethyl acetate, and dilute HCl. The filtrate was diluted with water, and the substance was extracted with ethyl acetate and chromatographed first in the benzene–acetone (10 : 1) system, with the isolation of a zone having R_f 0.62-0.73, and then in the ethyl acetate–hexane (1 : 3) system, with the isolation of a zone having R_f 0.49-0.60. This gave 122 mg (63%) of 12a-deoxychromocyclin, $C_{22}H_{10}O_8$ (V); mp 284-286°C (from ethyl acetate); $[\alpha]_D^{24}$ +860° (c 0.1; acetone); mol. wt. 412; λ_{max} 240, 289, 337, 374, 443, 476 nm (log ε 4.45; 4.19; 3.98; 4.26; 4.49; 4.32); ν_{max} 1595, 1645, 3380, 3550 cm^{-1}.

5. 2-Acetyl-2-decarboxamido-4-dedimethylaminotetracycline (XI). A solution of 50 mg of a crude preparation of the hydrochloride of 2-acetyl-2-decarboxamidotetracycline (X), obtained by a method described previously from the butanolic mother solution in the isolation of tetracycline from S. aureofaciens [10], in 8 ml of tetrahydrofuran was mixed with 2 ml of a 50% ethanolic solution of triethylamine, and the mixture was evaporated. The residue was dissolved in 8 ml of tetrahydrofuran, 4 ml of MeI was added, and the mixture was left at 20°C for a week. The solvents were separated off, 2 ml of acetic acid and 40 mg of activated Zn dust were added, the mixture was stirred for 15 min and filtered, and the filtrate was evaporated. The residue was dissolved in a mixture of ethyl acetate and 0.1 N HCl, and the aqueous layer was additionally extracted with ethyl acetate. The extracted substance was chromatographed in the ethyl acetate–hexane (1:1) system, giving 14 mg of dedimethylaminotetracycline (R_f 0.53) and 4 mg of compound (IX) with R_f 0.71, λ_{max} 222, 274, 360, 418 nm (log ε 4.26; 4.27; 4.16; 4.02); m/e 382 – (M–18).

6. 2-Acetyl-2-decarboxamido-4-dedimethylamino-5a,6-anhydrotetracycline (XIII). A solution of 2.2 mg of compound (XI) in 0.1 ml of methanol and 2 ml of 2 N HCl was boiled for 5 min and, after cooling, it was diluted with water and the substance was extracted with ethyl acetate and was chromatographed in the benzene–acetone (10:1) system. This gave 1.4 mg (67%) of the anhydro compound, $C_{21}H_{18}O_7$ (XIII) with R_f 0.79; mol. wt. 382; λ_{max} 224, 271, 424 nm (log ε 4.34; 4.58; 3.82).

SUMMARY

1. The glycoside chromocyclomycin has been isolated from the culture liquid of Streptomyces LA-7017, and its aglycone, chromocyclin, has been obtained.

2. As a result of degradation and the results of a comparison with the properties of model compounds, structure (I) has been established for chromocyclin.

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