OLIVOMYCIN AND RELATED ANTIBIOTICS

XXXI. STEREOCHEMISTRY OF CHROMOCYCLIN

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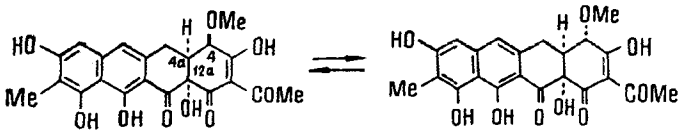
In a preceding communication we described how the structure of chromocyclin – the tetracyclic aglycone of the glycoside chromocyclomycin produced by Streptomyces LA-7017 – was determined [1]. Continuing this work, we have studied the stereochemistry of chromocyclin and have established that it possesses the absolute configuration (I).



I II

The spatial arrangement of the substituents at the C_4 and C_{4a} asymmetric centers of chromocyclin was determined with the aid of NMR spectra. It was found that when the spectrum was measured in pyridine solution the signal of the H_4 proton – which was located at 5.05 ppm, J = 4 Hz, immediately after the dissolution of the chromocyclin in pyridine – began to weaken and in addition to it a doublet appeared at 4.27 ppm with J = 12 Hz, increasing in intensity (Fig. 1). Simultaneously, the three-proton singlet of the methoxy group split into two with the appearance of a component in the stronger field. In the equilibrium state, which was reached after 1 h, the relative intensities of the original and the newly appeared signals were almost the same. The spectral changes show a reversible epimerization of chromocyclin at the C_4 asymmetric center (I ⇌ II), the values of $J_{4,4a}$ showing that in the 4-epichromocyclin formed the H_4 and H_{4a} protons are located trans-diaxially, and the initial chromocyclin has the H_4,H_{4a}-cis-ea configuration. This conclusion is confirmed by the diamagnetic shift of the H_4 signal in the epimerization of chromocyclin (greater shielding of the axial proton as compared with the equatorial), and agrees well with literature information on the NMR spectra of the anhydrotetracyclines and their 4-epimers [2].

We established the relative configurations of the asymmetric centers of C_{4a} and C_{12a} in the following way. It has been shown previously for a number of compounds that the acetylation of a hydroxy group may cause a paramagnetic shift of a proton of the neighboring carbon atom, the magnitude of this shift depending on the mutual positions of the hydroxyl and the proton: it is considerable for cis compounds (1.0-1.2 ppm with a-OH : e-H and 0.3-0.5 ppm for e-OH : a-H) but is close to zero if the hydroxyl is in the trans position to the proton (e-OH : e-H or a-OH : a-H) [3].

III IV

The results of a comparison of the NMR spectra of chromocyclin (I) and its 8,10,12a-triacetate (Fig. 2) have shown that the acetylation of the C_{12a} hydroxy group causes a considerable paramagnetic shift of

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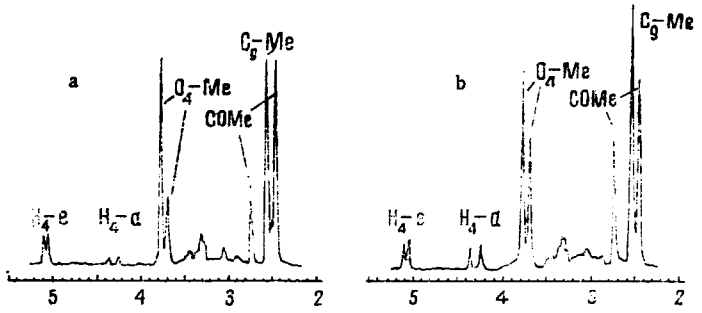


Fig. 1. NMR spectrum of chromocyclin (I) in pyridine solution 10 min (a) and 1 h (b) after dissolution.

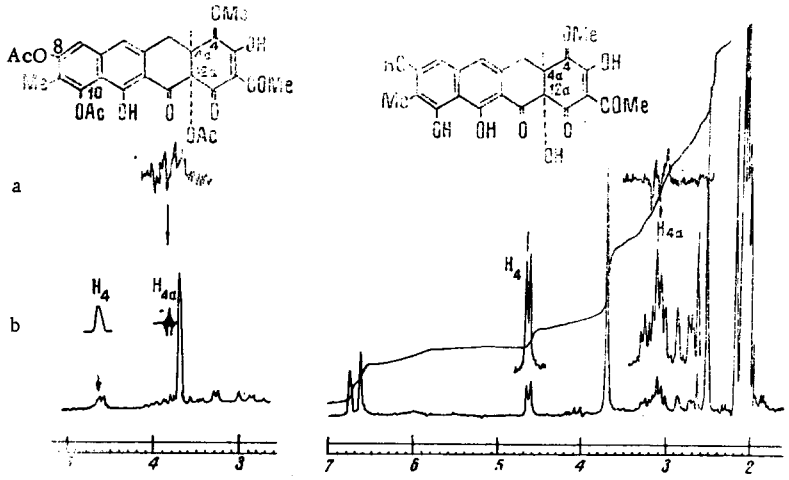


Fig. 2. NMR spectra of chromocyclin (I) and its 8,10,12a-triacetate in deuteroacetone (a – INDOR from H_4; b – double NMR on irradiated H_{4a}).

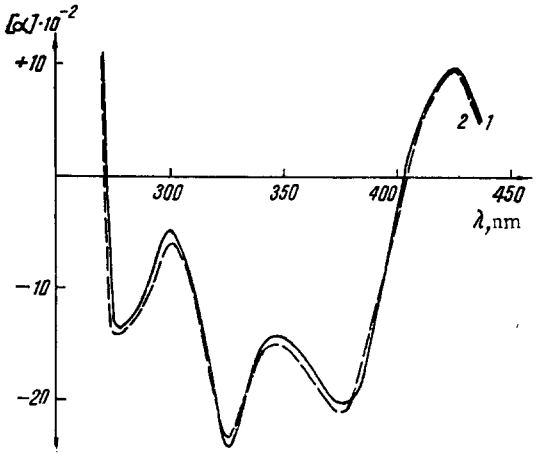
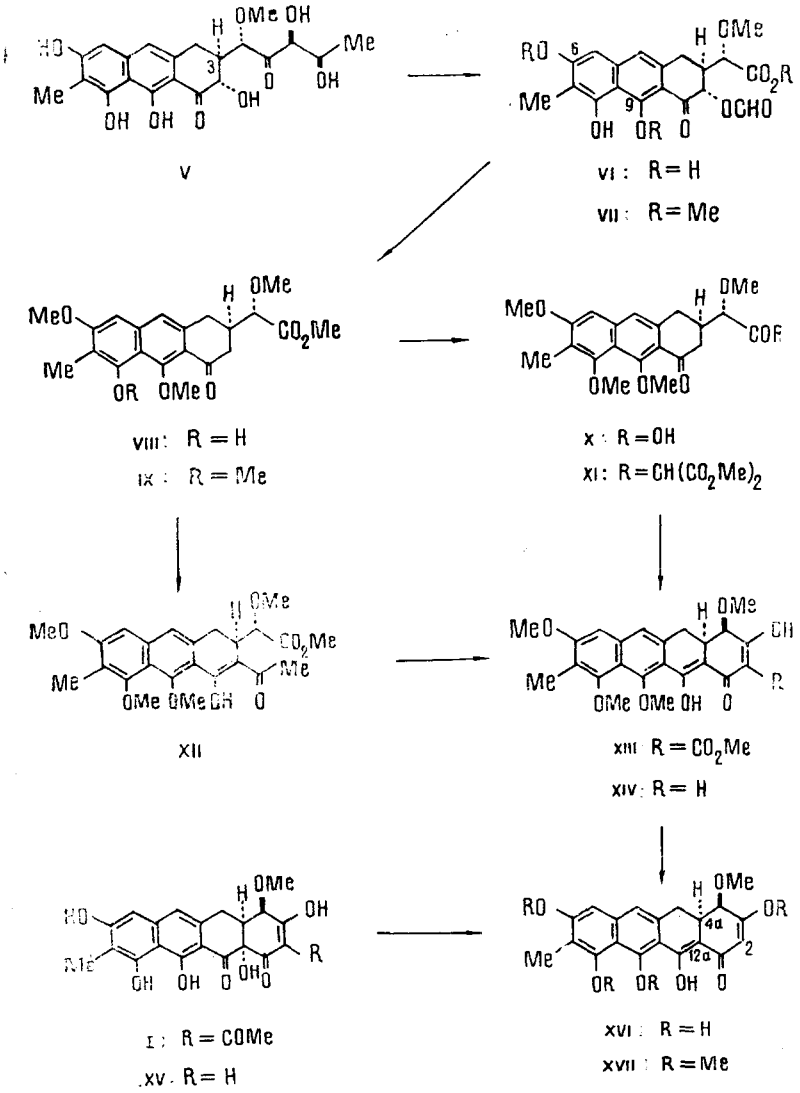


Fig. 3. ORD curves of 3,8,10,11-tetra-O-methyl-2-deacetyl-12a-deoxychromocyclin (XVII) obtained by the degradation of chromocyclin (I) and by partial synthesis from chromomycinone (2).

the H_{4a} signal ($\Delta\delta$ 0.75 ppm), which shows the cis position of the angular proton and hydroxyl. Thus, chromocyclin possesses the relative configuration cis,cis-4H,4aH,12aOH.

The absolute configuration of the asymmetric center at C_4 and, hence, of the whole chromocyclin molecule was determined from the change in the optical activity on C_4-epimerization. It was found that the conversion of chromocyclin (I) into 4-epichromocyclin (II) is accompanied by an optical shift ($\Delta[M]_D + 2300°$),* the magnitude of which is similar, although the sign is the opposite, to the change in the molecular rotation on passing from anhydrotetracycline (III) to its 4-epimer (IV) ($\Delta[M]_D - 2340°$ [4]). This shows the 4β position of the methoxyl in chromocyclin, which has the absolute configuration 4R,4aS,12aS (I).



For a definitive proof of the structure and stereochemistry of chromocyclin, we performed a partial synthesis of the tetramethyl ether of its 2-deacetyl-12a-deoxy derivative (XVII). For this purpose, chromomycinone (V) was oxidized with periodate to formylchromomycinonic acid (VI) (compare the periodate oxidation of olivin [5]), which forms the trimethyl ether/ester (VII) under the action of diazomethane. This substance was reductively deformyloxylated (VII → VIII) and was then methylated with methyl iodide in the presence of K_2CO_3 in dimethylformamide. The ester (IX) obtained was saponified to the free acid (X), the chloride of which was condensed with methoxymagnesiomalonic ester with the formation of the acylmalonate

* The molecular rotation of 4-epichromocyclin (II), which was isolated in the individual state, was calculated from information on the mutarotation of chromocyclin (I) in pyridine solution: $[\alpha]_D^{init} -350° \rightarrow [\alpha]_D^{1h} -109°$. According to the NMR spectrum (Fig. 1), after 1 h, the mixture contains about 55% of chromocyclin (I) and 45% of 4-epichromocyclin (II). Hence, 4-epichromocyclin (II) has $[M]_D = 0.01\ M \times ([\alpha]_D^{1h} - 0.55\ [\alpha]_D^{init})/0.45 = +800°$.

(XI). On the action of sodium methylsulfinylmethylide in dimethyl sulfoxide, this substance gave a low yield of a mixture of the tetracyclic ester (XIII) and the product of its demethoxycarbonylation (XIV).

A more effective method of constructing the fourth ring is the C-acylation of the ester (IX) with phenyl acetate in the presence of sodium methylsulfinylmethylide. As a result of this reaction we obtained in addition to the methyl ketone (XII) the product of its cyclization (XIV), which was then converted by the action of diazomethane into the methyl ether (XVII). In addition, chromocyclin was subjected to acid cleavage (I → XV) and then to reduction with Zn + AcOH to 2-deacetyl-12a-deoxychromocyclin (XVI) (see [1]). The product of the methylation of this substance with diazomethane and compound (XVII) obtained by partial synthesis proved to be identical according to chromatography and UV and mass spectroscopy, and also from their spectropolarimetric characteristics in the 250-450 nm range (Fig. 3). Since the C_3 asymmetric center of chromomycinone (V) is not affected in the process of synthesizing (XVII) from (V), this synthesis is an unambiguous proof not only of the structure but also of the absolute configuration of chromocyclin (I).

EXPERIMENTAL

For general information on the experimental work, see [1].

1. Mutarotation of Chromocyclomycin and Chromocyclin in Pyridine Solution. Chromocyclomycin: $[\alpha]_D^{27}$ −209° (after 1.5 min), −188° (after 10 min), −150° (after 30 min), +21° (after 8 h; times given from the moment of dissolution) (c 0.5; pyridine). Chromocyclin: $[\alpha]_D^{27}$ −350° (after 1.5 min), −310° (after 10 min), −233° (after 30 min), −109° (after 1 h and more) (c 0.3; pyridine).

2. Chromocyclin Acetates. A solution of 300 mg of chromocyclin (I) in 5 ml of Ac_2O was boiled for 2 h and evaporated, and the residue was chromatographed in the benzene–acetone (10:1) system. The zone with R_f 0.49-0.54 yielded 89 mg (25%) of chromocyclin diacetate, $C_{26}H_{26}O_{11}$; mp 152-154°C (from ethanol); $[\alpha]_D^{20}$ −130° (c 0.2; chloroform); mol. wt. 512; λ_{max} 224, 272, 420 nm (log ε 4.41; 4.67; 3.94); ν_{max} 1585, 1635, 1690, 1760, 3380 cm^{-1}.

The zone with R_f 0.43-0.45 yielded 283 mg (73%) of chromocyclin 8,10,12a-triacetate, $C_{28}H_{28}O_{12}$; mp 160-162°C (from ethanol); $[\alpha]_D^{20}$ −255° (c 0.15; chloroform); mol. wt. 554; λ_{max} 225, 272, 305sh, 392 nm (log ε 4.44; 4.67, 4.17, 3.86); ν_{max} 1585, 1635, 1690, 1760, 3380 cm^{-1}.

3. 2-Formylchromomycinonic Acid (VI). To a solution of 319 mg of chromomycinone (V) in 15 ml of methanol cooled to −15°C was added 72 ml of a 0.035 M solution of $NaIO_4$ cooled to 0°C. The reaction mixture was kept in the dark at room temperature for 5 min, and the substance was extracted with ethyl acetate and was chromatographed in the benzene–acetone (3:2) system. The zone with R_f 0.46-0.50 yielded 150 mg (51%) of an acid $C_{19}H_{18}O_9$ (VI); $[\alpha]_D^{25}$ −98° (c 0.3; ethanol); mp 221-222°C (from acetonitrile); mol. wt. 390; λ_{max} 232, 282, 325, 340, 415 nm (log ε 4.37; 4.57; 3.83; 3.86; 3.88); ν_{max} 1160, 1465, 1535, 1635, 1690, 1734 cm^{-1}.

4. Methyl 2-Formyl-6,9-dimethylchromomycinonate (VII). The acid (VI) (200 mg) in 2 ml of ethanol was methylated with 8 ml of a 0.6 M ethereal solution of CH_2N_2 (2 h at 20°C) and was then chromatographed in the benzene–acetone (15:1) system. The zone with R_f 0.50-0.62 gave 60 mg (27%) of the ester $C_{22}H_{24}O_9$ (VII), $[\alpha]_D^{20}$ −6° (c 0.2; ethanol); mol. wt. 432; λ_{max} 224, 278, 327, 340, 391 nm (log ε 4.36; 4.69; 3.83; 3.79; 3.76); ν_{max} 1570, 1605, 1635, 1690, 1754, 1755sh, 3330 cm^{-1}.

The zone with R_f 0.31-0.39 yielded 66 mg (30%) of methyl 2-formyl-6-methylchromomycinonate, $C_{21}H_{22}O_8$; mp 159°C (from ethanol); mol. wt. 418; λ_{max} 224, 278, 326, 335 nm (log ε 4.0*; 4.64; 3.66; 3.89); ν_{max} 1570, 1628, 1680, 1752, 3320 cm^{-1}.

The zone with R_f 0.41-0.49 yielded 57 mg (28%) of methyl 6,9-dimethylchromomycinonate, $C_{21}H_{24}O_8$; mp 170°C (from ethanol); mol. wt. 404; λ_{max} 224, 279, 327, 340, 390 nm (log ε 4.35; 4.67; 3.81; 3.74; 3.73); ν_{max} 1570, 1605, 1635, 1680, 1745, 3280, 3430 cm^{-1}.

5. Methyl 6,9-Dimethyl-2-deoxychromomycinonic Acid (VIII). A solution of 70 mg of the ester (VII) in 2 ml of 85% formic acid was stirred with 100 mg of activated Zn dust for 20 min and was then filtered and diluted with water. The substance was extracted with ethyl acetate and chromatographed in the benzene–acetone (20:1) system. The zone with R_f 0.60-0.78 yielded 42 mg (67%) of a hydrogenolysis product with the composition $C_{21}H_{24}O_7$ (VIII); $[\alpha]_D^{20}$ −19° (c 0.25; ethanol); mol. wt. 388; λ_{max} 224, 278, 325, 340sh, 388 nm (log ε 4.34; 4.68; 3.84; 3.81; 3.78); ν_{max} 1605, 1635, 1680, 1750, 3310 cm^{-1}.

*As in Russian original – Publisher.

6. Methyl 6,8,9-Trimethyl-2-deoxychromomycinonate (IX). A mixture of 50 mg of the 6,9-dimethyl derivative (VIII), 0.3 ml of methyl iodide, and 200 mg of K_2CO_3 in 1 ml of 90% dimethylformamide was stirred at 20°C for 7 h and was then diluted with water and extracted with chloroform. The extracted substance was chromatographed in the benzene–acetone (10:1) system. The zone with R_f 0.49-0.61 yielded 42 mg (82%) of the 6,8,9-trimethyl derivative $C_{22}H_{26}O_7$ (IX); $[\alpha]_D^{20}$ −56° (c 0.5; ethanol); mol. wt. 402; λ_{max} 227, 268, 329, 360sh nm (log ε 4.21; 4.66; 3.94; 3.64).

7. 6,8,9-Trimethyl-2-deoxychromomycinonic Acid (X). The ester (IX) (18 mg) in 2 ml of methanol was hydrolyzed with 2 ml of 0.2 N KOH (15 min at 20°C), and the reaction mixture was brought to pH 3 with 0.1 N H_2SO_4 and was extracted with ethyl acetate. After chromatography in the benzene–acetone (5:1) system, the zone with R_f 0.35-0.47 yielded 12.2 mg (71%) of the acid $C_{21}H_{24}O_7$ (X); mol. wt. 388; λ_{max} 226, 267, 329, 360sh nm (log ε 4.18; 4.68; 3.94; 3.64).

8. Dimethyl 6,8,9-Trimethyl-2-deoxychromomycinonoylmalonate (XI). At −70°C, 42 mg of PCl_5 was added to 79 mg of the acid (X), 21 mg of triethylamine, and 170 mg of dimethylformamide in 3 ml of tetrahydrofuran. The mixture was stirred at −70°C for 30 min and at 20°C for 1 h, and was then evaporated and the residue was dried at 40°C/0.1 mm. The acid chloride so obtained was dissolved in 2 ml of tetrahydrofuran and was added at −70°C to 2.2 ml of a 0.09 M tetrahydrofuran solution of the MeOMg derivative of dimethyl malonate. The mixture was stirred at −70°C for 30 min and then at 20°C for 1 h and was acidified with acetic acid, diluted with water, and extracted with ethyl acetate. After chromatography in the benzene–acetone (10:1) system, a zone with R_f 0.33-0.41 yielded 48 mg (46%) of the acylmalonate $C_{26}H_{30}O_9$ (XI); mol. wt. 502; λ_{max} 228, 276, 325 nm (log ε 4.30; 4.72; 3.98); ν_{max} 1620, 1680, 1730, 1765 cm^{-1}.

9. 2-Methoxycarbonyl-8,10,11-trimethyl-2-deacetyl-12a-deoxychromocyclin (XIII). At 20°C, a solution of 35 mg of the acylmalonate (XI) in 1 ml of dimethyl sulfoxide was added to a solution of $MeSOCH_2Na$ obtained from 10 mg of NaH and 0.5 ml of dimethyl sulfoxide. The mixture was kept at 20°C for 15 min and at 60°C for 10 min and was then acidified with acetic acid, diluted with water, and extracted with ethyl acetate. The extract was washed with water from dimethyl sulfoxide and was evaporated, and the residue was chromatographed in the benzene–acetone (5:1) system. A zone with R_f 0.67-0.73 yielded 2.5 mg of the cyclization product $C_{25}H_{26}O_9$ (XIII); mol. wt. 470; λ_{max} 234, 262, 387, 395 nm (log ε 4.34; 4.39; 4.08; 4.02); ν_{max} 1590, 1610, 1735 cm^{-1}.

A zone with R_f 0.82-0.85 yielded 0.7 mg of compound $C_{23}H_{24}O_7$ (XIV); mol. wt. 412; λ_{max} 234, 262, 287, 395 nm (log ε 4.35; 4.40; 4.15; 4.08).

10. Methyl 2-Acetyl-6,8,9-trimethyl-2-deoxychromomycinonic Acid (XII). To a solution of 100 mg of the oxo ester (IX) in 1 ml of benzene freed from oxygen by boiling in a current of argon was added 35 mg of NaH, and the mixture was stirred at 20°C for 30 min. Then 100 mg of phenyl acetate in 1 ml of benzene was added and the resulting mixture was boiled for 2.5 h. It was decomposed with acetic acid, diluted with water, and extracted with ether. After chromatography in the benzene–acetone (20:1) system, a zone with R_f 0.55-0.64 yielded 45.5 mg (41%) of the C-acetate $C_{21}H_{28}O_8$ (XII); mol. wt. 444, $[\alpha]_D^{20}$ +110° (c 0.07; ethanol); λ_{max} 231, 268, 302, 316, 366 nm (log ε 4.40; 4.34; 3.93; 3.95; 4.35); ν_{max} 1545, 1575, 1624, 1750 cm^{-1}; δ^{CDCl_3} 2.34 (3H, s., COMe).

11. 8,10,11-Trimethyl-2-deacetyl-12a-deoxychromocyclin (XIV). The oxo ester (IX) was condensed with phenyl acetate under the conditions of experiment 10 for 5 h. After chromatography, a zone with R_f 0.54-0.65 yielded 21 mg (12%) of the C-acetate (XII), and a zone with R_f 0.47-0.52 yielded 20 mg (15%) of the cyclization product $C_{23}H_{24}O_7$ (XIV); mol. wt. 412; λ_{max} 234, 260, 386, 395 nm (log ε 4.39; 4.42; 4.27; 4.26).

12. 2-Deacetyl-12a-deoxychromocyclin (XVI) and the Products of Its Methylation with Diazomethane.

A. Under the conditions described previously [1], 325 mg of 2-deacetylchromocyclin (XV) was reduced with zinc in acetic acid, and the product was chromatographed in the benzene–acetone (5:1) system. This gave 110 mg (34%) of the starting material (XV), with R_f 0.35, and 95 mg (31%) of the deoxy compound $C_{20}H_{18}O_7$ (XVI); mol. wt. 370; mp 228-230°C (from ethyl acetate); $[\alpha]_D^{20}$ +425° (c 0.25; acetone); R_f 0.59; λ_{max} 236, 267, 343; 435, 459 nm (log ε 4.33, 4.44; 3.74; 4.38; 4.40); ν_{max} 1585, 1620, 1720, 3885 cm^{-1}.

B. To a solution of 100 mg of compound (XVI) in 2 ml of methanol was added 15 ml of a 0.5 M ethereal solution of CH_2N_2, and after 2 h the mixture was evaporated. The resulting mixture of the dimethyl and trimethyl ethers was separated by chromatography in the benzene–acetone (8:1) system. From a zone with R_f 0.37-0.44 was obtained 12.5 mg (12%) of the dimethyl ether $C_{22}H_{16}O_7$, mp 221-223°C (from ethanol); λ_{max} 224, 258, 281, 332, 420 nm (log ε 4.32; 4.37; 4.20; 3.92; 4.39); ν_{max} 1465, 1510, 1590, 1630, 3330 cm^{-1}.

A zone with R_f 0.61-0.68 yielded 43 mg (39%) of the trimethyl ether $C_{23}H_{18}O_7$; mp 198.5-200°C (from ethanol); mol. wt. 492; λ_{max} 228, 260, 327, 404, 415sh nm (log ε 4.37; 4.46; 3.87; 4.43; 4.41); ν_{max} 1475, 1510, 1590, 1625, 3335 cm^{-1}.

13. 3,8,10,11-Tetramethyl-2-deacetyl-12a-deoxychromocyclin (XVII). A. The mixture of dimethyl and trimethyl ethers obtained in the preceding experiment (40 mg) was dissolved in 2 ml of 90% dimethylformamide, and 0.4 ml of methyl iodide and 50 mg of K_2CO_3 were added and the mixture was stirred at 20°C for 7 h. Then it was diluted with water, and the product was extracted with chloroform and chromatographed in the benzene–acetone (20:1) system. A zone with R_f 0.40-0.48 yielded 7 mg of the tetramethyl ether $C_{24}H_{26}O_7$ (XVII); mol. wt. 426; $[\alpha]_D^{20}$+47° (c 0.7; ethanol); λ_{max} 226, 255, 315, 395 nm (log ε 4.36; 4.39; 3.76; 4.27); ORD: $[\alpha]_{425}$+950°, $[\alpha]_{370}$−2060°, $[\alpha]_{345}$−1740°, $[\alpha]_{325}$−2480°, $[\alpha]_{300}$−430°, $[\alpha]_{285}$−1230° (c 0.05; ethanol).

B. The trimethyl ether (XIV) (expt. 11) (6 mg) in 0.1 ml of methanol was methylated with 2 ml of a 0.7 M solution of CH_2N_2 in ether (2 h at 20°C) and the product was chromatographed in the benzene–acetone (20:1) system. A zone with R_f 0.40-0.46 yielded 1.4 mg of a substance which was identical in chromatographic mobility, UV spectrum, mass spectrum, and ORD curve (see Fig. 3) with the tetramethyl ether (XVII) from expt. 13A.

SUMMARY

1. The relative and absolute configurations of chromocyclin (I) have been established by NMR spectroscopy and polarimetry.

2. The structure and stereochemistry of chromocyclin (I) have been shown by the partial synthesis [starting from chromomycinone (V)] of its degradation product (XVII).

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