52. The Structure of Chromomycin A₃,^{*)} a Cancerostatic Antibiotic

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Chromomycin is the name given to a group of cancerostatic and anticancer antibiotics produced by *Streptomyces griseus* No. 7, its discovery and isolation being accomplished by Nakazawa *et al.*, in 1955.¹⁾ The principal one which was named chromomycin A₃ was then isolated by Tatsuoka and Mizuno²⁾ in Takeda Laboratories.

Chromomycin A₃ is now being sold as an anti-tumor drug in this country under the name of Toyomycin.³⁾ Similar compounds reported by other groups are: aureolic acid,⁴⁾ M5-18903,⁵⁾ mithramycin,⁶⁾ and NSCA-649,⁷⁾ all in the United States, olivomycin⁸⁾ in U. S. S. R., LA-7017⁹⁾ in Italy, and aburamycin¹⁰⁾ in Japan. These antibiotics apparently belong to a new type of compounds, but their structures remained unclarified.

Chromomycin A₃ is a yellow pigment susceptible to both acid and alkali. During the early stage of our studies, all efforts to obtain a crystalline degradation product as crystals or attempts to determine its molecular weight by physical or physico-chemical means were unsuccessful.

It was finally discovered, however, that chromomycin A₃ could be hydrolyzed with 50% acetic acid to give lipid-soluble and water-soluble fractions. From the lipid-soluble fraction a yellow crystalline substance, chromomycinone, containing a saturated carbonyl group, was isolated; the elementary analysis of this substance and the molecular weight measured by the osmotic pressure method were in agreement with C₂₁H₂₄O₉.

Chromomycinone exhibits a UV-spectrum ($\lambda_{\text{max}}^{\text{EtOH}}$: 232 (22,000), 282 (37,000), 326 (7,000), 340 (7,000), 415 (10,000)), which is almost identical with that of chromomycin A₃. This indicates that chromomycinone retains the original chromophore of chromomycin A₃ and that the molecular weight of chromomycin A₃ is about 1000.

By repeated chromatography of the water-soluble fraction we

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were able to isolate four new sugars as crystals. These were named chromose A, B, C, and D. They were susceptible toward acid and alkali, however, their acetates and methylglycosides were prepared and the structures determined on the basis of chemical and NMR-studies. The structure of one of the sugars, chromose A, has already been reported.¹¹⁾ The remaining three were also investigated by similar fashions, and in conjunction with the sign of rotation, these four sugars can now be summarized in Fig. 1.

The Four Chromoses

Fig. 1

Acetylation of chromomycinone gives a tetraacetate, a pentaacetate, a hexaacetate, and anhydrochromomycinone pentaacetate according to the reaction conditions.

Treatment of chromomycinone with acetone and boron-trifluoride gives an acetonide, which on acetylation furnishes the triacetate and tetraacetate. These results demonstrate that six hydroxyls are present in chromomycinone.

Furthermore, careful inspection of NMR- and IR-spectra of these compounds led us to the speculation that the following functional groups are present in the chromomycinone molecule: $\text{H}_3\text{C}-\text{CH}<$, 3 OH (aliphatic), 3 OH (phenolic), OCH_3 (aliphatic), 2 $\text{C}=\text{O}$ (one saturated and the other conjugated), CH_3 (aromatic), and two aromatic protons.

Zinc dust distillation of chromomycinone was then conducted and the product was characterized as a mixture of 2-methylantracene and anthracene, and this indicated the nature of the skeleton. The skeleton of chromomycinone was further clarified by the finding that the UV-spectrum of chromomycinone is of close similarity to that of anhydroterracycline,¹²⁾ which is known in the tetracycline chemistry.

The part structure proposed in Fig. 2 receives further support by the synthesis of a model compound, 8,9,10-trihydroxy-1-oxo-1,2,3,

4-tetrahydroanthracene,¹²⁾ and comparison of the NMR-, IR-, and UV-spectra with those of chromomycinone.

Another evidence for the part structure is that NMR-spectra of both chromomycinone and the model compound clearly demonstrate characteristic signals at 15 and 10 ppm, which disappear upon addition of D₂O and D₂SO₄ to the sample cell.

An alternative degradation started with the acetate of chromomycin A₃:

Acid hydrolysis of the acetate in a similar fashion as in the case of chromomycin A₃ furnished a lipid-soluble fraction, chromose A acetate, and chromose B, C, and D.

The lipid-soluble fraction failed to be crystallized by any means, however, a homogeneous substance was separated by chromatography and the NMR-spectrum of this compound definitely demonstrated that this was a tri-acetate. Sodium borohydride reduction of this triacetate under drastic conditions followed by air-oxidation in alkali furnished a crystalline substance in poor yield. This new compound, the second key degradation product, revealed an intense blue coloration with magnesium acetate, which is known as characteristic of hydroxyquinones. The compound, therefore, was named "chromomyciquinone".

The assumption that the compound is a hydroxyquinone was confirmed by the reductive acetylation to an octaacetate, which showed a typical UV-spectrum of naphthalenes.

A number of reactions were undertaken with chromomyciquinone

and several derivatives were submitted to detailed NMDR studies. These results, of particular, NMDR^{*)} and NMTR^{**)} studies carried out with the tetraacetate of chromomyciquinone acetoneide led us to the assignment structure (III) for this compound (Fig. 3).

Fig. 3

Efforts were then directed toward the correlation of chromomycinone with chromomyciquinone. Catalytic hydrogenation of chromomycinone (I) gave a cyclic tetrahydrochromomycinone (II), which in turn was subjected to reduction with sodium borohydride and air-oxidation in alkali to give chromomyciquinone (III) in 60% over-all yield. These reactions are shown in Fig. 3, and the structure (I) was assigned to chromomycinone.

The structure (I) receives further support by that chromomycinone hexaacetate gives anhydrochromomycinone pentaacetate (IV) on treatment with pyridine.

It is of interest to note that chromomycinone might be formed biogenetically by the cyclization of a polyacetate precursor, the cyclization being accompanied by a decarboxylation and introduction of three oxygens and one carbon.

The position of attachment of the sugar residue was determined as follows:

- 1) The sugar can not be present on the hydroxyl at C8 or C9 because chromomycin A₃ exhibits 15- and 10-ppm signals.
- 2) The sugar can not be present on any of three hydroxyls at C8, C3', and C4', because cleavage of chromomycin A₃ acetate gave a chromomycinone triacetate in which these remained acetylated.
- 3) A choice between the remaining two hydroxyls, namely C2 and C6, was made by comparing the UV-spectra of chromomycin A₃ acetate and chromomycinone hexaacetate.

^{*)} Nuclear magnetic double resonance.

^{**)} Nuclear magnetic triple resonance.

Finally, the sequence of the sugars was deduced by partial hydrolysis of the acetate of chromomycin A₃ in 60% propionic acid; the hydrolysis splitted chromose A in the acetate form and chromose B and C in the free form. On much milder hydrolysis, chromose A and chromose B were detected in the hydrolysate and glycosides, chromomycinone-chromose D and chromomycinone-chromose D-chromose C, were isolated.

From these results we propose the structure (V) for chromomycin A₃.

Chromomycin A₃

(V)

Fig. 4

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