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THE FULL STRUCTURES OF THREE CHROMOMYCINS, A_2, A_3 and A_4

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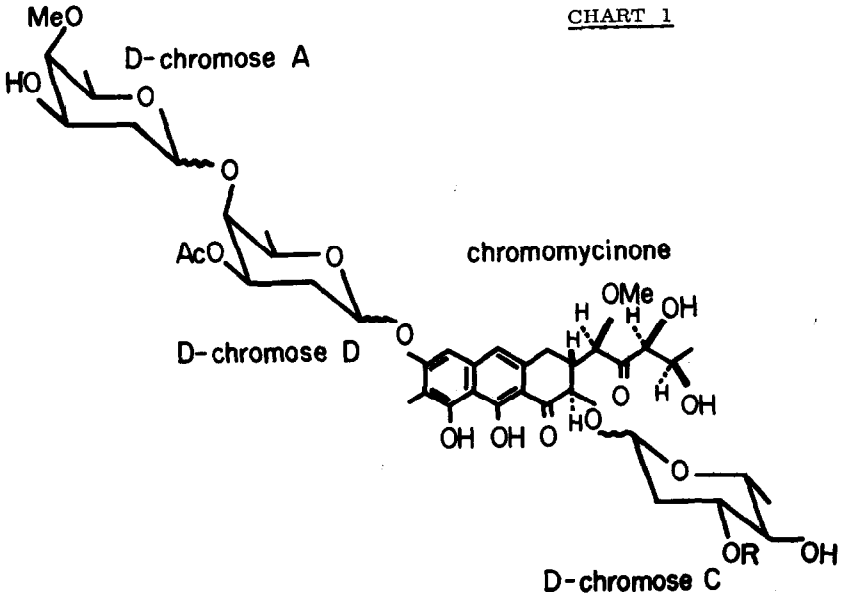
The chromomycins are a group of cancerostatic antibiotics produced by Streptomyces griseus[1] and are undoubtedly closely related to olivomycin[2] and several other antibiotics[3] isolated by various groups. The principal constituent, chromomycin A_3 (1) is commercially available[4], while chromomycin A_2 (2) and chromomycin A_4 (3) are the major by-products isolated during the manufacture of A_3. The structures of these three constituents are reported in this communication.

The three chromomycins[5] can be isolated by silica gel chromatography of the chromomycin A mixture using ethyl acetate containing 1% oxalic acid as the solvent, the ratio of A_2, A_3 and A_4 being roughly 1 : 8 : 1.

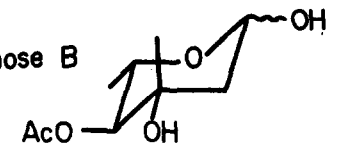
1] Chromomycin A_3 (1)

A planar structure 4 had previously been derived[6] for chromomycin A_3 on grounds of a "tetrasaccharide" that was isolated from the alkaline hydrolysate of its "methyl ether," m. p. 185°. However, subsequent

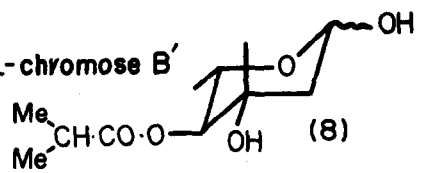
CHART 1



(1) chromomycin A_3 R: L-chromose B



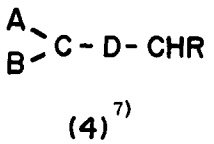
(2) chromomycin A_2 R: L-chromose B′



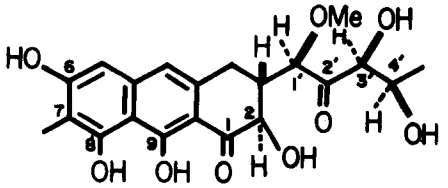
7) CHR-D-C-A ≡ chromomycin A_4 (3) R: H

6) monodeacetylchromomycin A_3 R: deacetylchromose B

investigations disclosed that the methyl ether, although crystalline, was a mixture of several constituents, and therefore the linkage and sequence of the four sugars, chromoses A[8) 9)], B[10)], C[10)] and D[10) 11)] were reinvestigated since the exact nature of the "tetrasaccharide" became dubious. This reinvestigation, together with elucidation of the full stereochemistry of the chromophore, chromomycinone[12)] (5) has now led to expression 1 (Chart 1).



(4)[7)]



(5)

Table 1. Chemical shifts of acetoxyl and C_7-Me peaks of chromomycinone (5) derivatives (ppm from internal TMS).

Position	C-9	C-8	C-6	C-2	C-3′	Me*	C-4′
Average	2.5	2.4	2.35	2.3	2.2	2.1	2.0
Hexaacetate	2.47	2.41	2.35	2.28	2.23	2.10	2.01
8, 3′, 4′-triacetate		2.41			2.21	2.05	1.97

* The aromatic methyl peaks are easily characterized because of their low height arising from coupling to the 5-H.

Table 2. Chemical shifts of carbinyl protons (ppm)

Position	2	3′	4′
Hexaacetate	5.57	5.28	5.42
8, 3′, 4′-triacetate	4.28	5.33	5.45

The points of attachment of sugar residues to chromomycinone were deduced in the following manner. An extensive comparison of the NMR acetoxyl peaks of thirteen chromomycinone derivatives acetylated at various positions revealed that the six acetoxyls, attached to C-9, C-8, C-6, C-2, C-3' and C-4', respectively, could be easily differentiated because each of these acetoxyl groups absorbed within a very narrow range characteristic of its position in the molecule (Table 1). Controlled hydrolysis of A_3-peracetate with 50% formic acid yielded a chromomycinone triacetate. The chemical shifts of the acetoxyl peaks of this triacetate (Table 1) unambiguously showed that positions C-9, C-6 and C-2 are unacetylated; the same conclusion is derived by comparing the chemical shifts of the carbinyl protons (Table 2), from which it is obvious that C_2-OH is unacetylated. These three positions indicate possible points of attachment, but C-9 can be excluded since the NMR spectrum of A_3 itself has two clear peaks at 9.79 and 15.75 ppm, which can be assigned to the C-8 and C-9 hydroxyls[6]. Thus the C-2 and C-6 are the only two possible positions for linkage of the sugars. In addition, it has already been shown that chromose D is attached to C-6[6].

Careful hydrolysis of A_3 with aqueous methanolic potassium carbonate yielded monodeacetylchromomycin A_3 (6) (Chart 1), which upon further hydrolysis with 50% acetic acid yielded CHR-D-C-A (7) (Chart 1)[7]. Since chromose B is not contained in this partial hydrolysis product it must constitute a terminal residue.

Methanolysis of A_3-pentatosylate (all hydroxyls excepting C_9-OH are tosylated; evidence from NMR) afforded the methyl glycosides of

chromose B, chromose D, deacetyl chromose D, 3-O-tosylchromose A[13] and 4-O-tosylchromose C[13]; production of 3-O-tosylchromose A indicates that chromose A must be at a terminal position, while production of 4-O-tosylchromose C indicates that of the two hydroxyl groups present in chromose C, at least the C_4-hydroxyl must have been free in the original antibiotic.

The above evidence reduces the number of possible structures for A_3 to the following four:

I. A-D-$^{2}_{6}$[CHR]-C-B

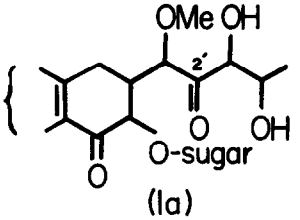
II. B-D-$^{2}_{6}$[CHR]-C-A

III. B-C-D-$^{2}_{6}$[CHR]-A

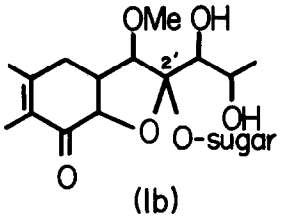
IV. A-C-D-$^{2}_{6}$[CHR]-B

The numerals, 2 and 6, indicate positions to which sugars are linked.

However, it was found that methanolysis of CHR-D-C-B pentatosylate (all hydroxyls excepting C_9-OH are tosylated) afforded the methyl glycosides of 4-O-tosylchromose C[13], 4-O-tosyldeacetylchromose D[13] and chromose B. This result can only be accounted for by sequence I. Finally, because a hemiketal is easily formed between the 2-OH and 2'-CO in chromomycinone derivatives[12], a further choice had to be made between the two possibilities Ia and Ib in sequence I.



(Ia)



(Ib)

However, Ib could be eliminated because the IR spectrum (KBr disk) of CHR-D-C-A (7) clearly showed three absorptions in the carbonyl region at 1738 (OAc of chromose D), 1728 (side-chain CO) and 1630 cm^{-1} (annular CO). The total structure of chromomycin A_3 is thus represented by 1 (corresponding to a molecular formula of $C_{51}H_{72}O_{23}$)[14]

2] Chromomycin A_2 (2)

Chromomycin A_2 was obtained as a yellow powder with $[\alpha]_D^{23}$ -61° (c 1.0 in ethanol), λ_{max}^{EtOH} mμ (log ε) 229 (4.37), 279 (4.67), 317 (3.86), 331 (3.75, 412 (3.89), and υ^{CHCl_3} 3400, 1730, 1715 (shoulder), 1630, 1205, 1065 cm^{-1}. The spectroscopic properties are very similar to chromomycin A_3 excepting that slight differences are detected in the NMR spectra. Namely, in the A_2 spectrum, the intensity of methyl peaks around 1.3 ppm is greater, and there is only one acetoxyl peak around 2 ppm (in contrast, A_3 has two acetoxyl peaks).

As mentioned above, careful hydrolysis of A_3 afforded monodeacetylchromomycin A_3 (6) (Chart 1). This same product (analyses, IR, UV, rotation) was obtained when A_2 was submitted to identical hydrolytic conditions. On the other hand, methanolysis of A_2 pentatosylate gave the methyl glycosides of deacetylchromose D, 3-O-tosylchromose A, 4-O-tosylchromose C, and chromose B′. Further hydrolysis of methyl chromoside B′ with 0.5 N sodium hydroxide gave methyl deacetylchromoside B and isobutyric acid, the latter being identified as its p-bromophenacyl ester. Thus, chromose B′ is 4-O-isobutyryldeacetylchromose B (8)[15], and chromomycin A_2 should be represented by structure 2 (molecular formula $C_{53}H_{76}O_{23}$)[14]

3] Chromomycin A_4 (3)

Chromomycin A_4 was obtained as a yellow powder with $[\alpha]_D^{21}$ -47° (c 1.0 in ethanol). The spectroscopic chromatographic and chemical properties were indistinguishable from those of CHR-D-C-A (7), $C_{42}H_{58}O_{19}$ [14], the partial hydrolysis product of A_3, and therefore the two are identical.

REFERENCES

1) S. Tatsuoka, A. Miyake and K. Mizuno, J. Antibiotics, Ser. B 13, 332 (1960); K. Mizuno, J. Antibiotics, Ser. A 16, 22 (1963).

2) M. G. Brazhnikova, E. B. Kruglyak, I. N. Kovsharova, N. V. Konstantinova and V. V. Proshlyakova, Antibiotiki, 7 39 (1962); Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, M. M. Shemyakin and M. G. Brazhnikova, Tetrahedron Letters, 1323, 3513 (1964).

3) References cited in reference 8.

4) "Toyomycin", Takeda Chemical Industries.

5) Although the chromomycins could not be obtained crystalline, all elementary analyses of chromomycins and derivatives were in good agreement with calculated values. The homogeneity of non-crystalline samples were checked by chromatography.

6) M. Miyamoto, K. Morita, Y. Kawamatsu, M. Sasai, A. Nohara, K. Tanaka, S. Tatsuoka, K. Nakanishi, Y. Nakadaira and N. S. Bhacca, Tetrahedron Letters, 2367 (1964).

7) CHR stands for the chromophore, chromomycinone [12], while A, B, C and D stand for the respective chromoses.

8) M. Miyamoto, Y. Kawamatsu, M. Shinohara, Y. Asahi, Y. Nakadaira, H. Kakisawa and K. Nakanishi, Tetrahedron Letters, 693 (1963).

9) J. S. Brimacombe, D. Portsmouth and M. Stacey, Chem. and Ind., 1758 (1964).

10) M. Miyamoto, Y. Kawamatsu, M. Shinohara, K. Nakanishi, Y. Nakadaira and N. S. Bhacca, Tetrahedron Letters, 2371 (1964).

11) J. S. Brimacombe and D. Portsmouth, Chem. and Ind., 468 (1965).

12) To be published .

13) Compared with authentic samples; details to be published.

14) Elementary analyses of chromomycins A_2, A_3 and A_4 showed that all three contained one mole of water.

15) Point of attachment of the isobutyryl group was easily disclosed by comparing the NMR spectra of chromose B, deacetylchromose B and chromose B'.