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CHROMOMYCIN A_2, A_3 and A_4

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THE Chromomycins are a group of cancerostatic and antitumor antibiotics produced by *Streptomyces griseus* No. 7[1]. They exhibit strong growth inhibition against Gram positive bacteria and are also effective against Ehrlich sarcoma, Yoshida sarcoma, tuberculous bacilli, etc. The principal constituent chromomycin A_3 is commercially available,[2] while chromomycin A_2 and chromomycin A_4 are the major byproducts isolated during the manufacture of A_3. In view of the close similarity in the UV spectra, the chromomycins are undoubtedly very closely related to other antibiotics such as aureolic acid,[3] aburamycin,[4] LA-7017,[5] M5-18903,[6,7] NSCA-649,[8] mithramycin,[9] olivomycin,[10] and antibiotic 3014.[11] Especially, the aglycone of mithramycin and antibiotic 3014 is identical[12] with the aglycone of the chromomycins, chromomycinone, the structure,[13] reactions[14] and stereochemistry (see 4)[15] of which have been clarified. Moreover, the structure of olivin,[16] the olivomycin aglycone, has recently

[1] M. Shibata, K. Tanabe, Y. Hamada, K. Nakazawa, A. Miyake, H. Hitomi, M. Miyamoto and K. Mizuno, *J. Antibiotics, Ser.* **B13**, 1 (1960); K. Mizuno, *Ibid.* **13**, 329 (1960); S. Tatsuoka, A. Miyake and K. Mizuno, *Ibid.* **13**, 332 (1960); K. Mizuno, *Ibid.* **13**, 335 (1960); K. Mizuno, *Ibid.* **A16**, 22 (1963).
[2] "Toyomycin", Takeda Chemical Industries.
[3] J. E Philip and J. H. Schenck, *Antibiot. and Chemoth.* **3**, 1218 (1953).
[4] H. Nishimura, R. Kimura, K. Tawara, K. Sasaki, N. Nakazima, N. Shimaoka, S. Okamoto, M. Shinohara and J. Isono, *J. Antibiotics, Ser.* **A10**, 205 (1957).
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[6] R. M. Gale, M. H. Hoehn and M. H. Cormick, *Antibiot. Ann.* **1958–1959**, 489 (1959).
[7] TLC has indicated that M5-18903 is roughly a 4:1 mixture of chromomycins A_3 and A_2. The authors are indebted to Dr. N. Neuss, Eli Lily Co., for a sample of M5-18903.
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[10] G. F. Gause, R. S. Ucholina and M. A. Sveshnikova, *Antibiotiki* **7**, 34 (1962); M. G. Brazhnikova, E. B. Kruglyak, I. N. Kovsharova, N. V. Konstantinova and V. V. Proshlyakova, *Ibid.* 39.
[11] R. S. Ucholina, E. B. Krugliak, V. N. Borisova, I. N. Kovsharova and V. V. Proshivakova, *Microbiologia* **34**, 147 (1965).
[12] M. G. Brazhnikova, E. P. Krugliak and A. S. Mensentev, *Antimicrobial Agents and Chemotherapy* 119 (1966).
[13] M. Miyamoto, K. Morita, Y. Kawamatsu, S. Noguchi, R. Marumoto, K. Tanaka, S. Tatsuoka, K. Nakanishi, Y. Nakadaira and N. S. Bhacca, *Tetrahedron Letters* 2355 (1964).
[14] M. Miyamoto, K. Morita, Y. Kawamatsu, S. Noguchi, R. Marumoto, M. Sasai, A. Nohara, Y. Nakadaira, Y. Y. Lin and K. Nakanishi, *Tetrahedron* **22**, 2761 (1966).
[15] M. Miyamoto, K. Morita, Y. Kawamatsu, K. Kawashima and K. Nakanishi, *Tetrahedron* **23**, 411 (1967).
[16] Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, M. M. Shemyakin and M. G. Brazhnikova, *Tetrahedron Letters* 1323 (1964).

been determined by Russian workers[17] and has been shown that the two aglycones, chromomycinone[18] and olivin[17] are closely related, i.e., olivin is 7-demethylchromomycinone. The sugars resulting from the hydrolysis of olivomycin[18,19] are again identical or closely related to the sugars obtained from the chromomycins, i.e. chromose A,[20,21] chromose B,[21,22] chromose B',[21,23] chromose C[21,22] and chromose D;[21,23] interestingly, all five are 2,6-dideoxy hexopyranoses, a fact that is biogenetically significant.[24] A full account on the structures of chromomycin A_3,[23,25] A_2[23] and A_4[23] is presented in this paper. The three chromomycins could be separated 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. The full structures, excepting the stereochemistry of the glycoside linkages, are summarized in Chart 1. The close similarity in the structures of chromomycinone, presumably derived from ten acetate units (as in 5), and the tetracyclines[26] (e.g. terramycin **6**,) mycinones[27] (e.g. ε-pyrromycinone or rutilantinone 7) and cervicarcin (**8**)[28] should be noted.

Chromomycin A_3 (**1**)

Chromomycin A_3 is composed of the chromophore, chromomycinone[13-15] (abbreviated to CHR) and the four chromoses, A, B, C and D. Hydrolysis with 50% aqueous acetic acid at 65° for 2 hr gave CHR, chromomycinone-chromose D (abbreviated to CHR-D), CHR-DCC and CHR-DCCB (see Experimental for estimation of mol. wts).

We had proposed for chromomycin A_3 a structure in which one mole each of the four chromoses are linked to chromomycinone as in 9.[23] Recent Russian papers on

[17] Yu. A. Berlin, O. A. Chuprunova, B. A. Klyashchitskii, M. N. Kolosov, G. Yu. Peck, L. A. Piotrovich, M. M. Shemyakin and I. V. Vasina, *Tetrahedron Letters* 1425 (1966).

[18] Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, M. M. Shemyakin and M. G. Brazhnikova, *Tetrahedron Letters* 3513 (1964).

[19] Yu. A. Berlin, S. E. Esipov, M. N. Kolosov and M. M. Shemyakin, *Tetrahedron Letters* 1431 (1966).

[20] M. Miyamoto, Y. Kawamatsu, M. Shinohara, Y. Asahi, Y. Nakadaira, K. Nakanishi and N. S. Bhacca, *Tetrahedron Letters* 693 (1963).

[21] M. Miyamoto, Y. Kawamatsu, M. Shinohara, Y. Nakadaira and K. Nakanishi, *Tetrahedron* **22**, 2785 (1966).

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

[23] M. Miyamoto, Y. Kawamatsu, K. Kawashima, M. Shinohara and K. Nakanishi, *Tetrahedron Letters* 545 (1966).

[24] H. Achenbach and H. Griesebach, *Z. Naturforschg.* **19b**, 561 (1964).

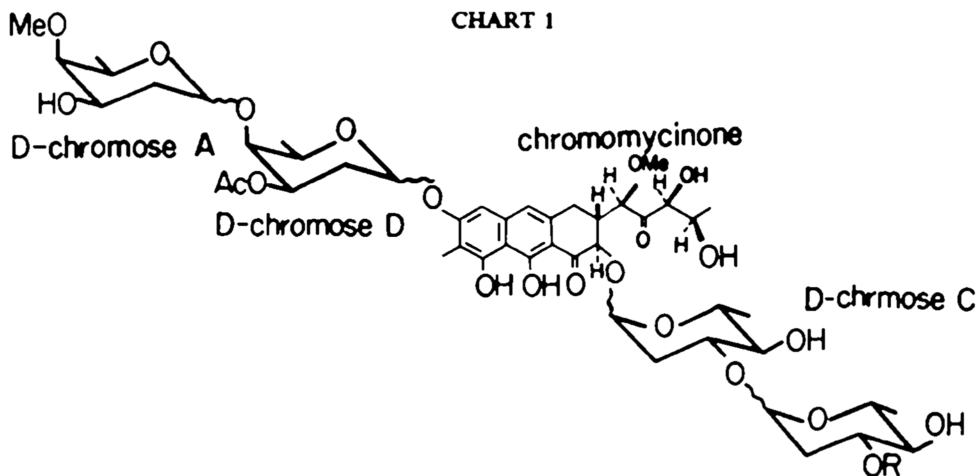
[25] S. Tatsuoka, K. Tanaka, M. Miyamoto, K. Morita, Y. Kawamatsu, K. Nakanishi, Y. Nakadaira and N. S. Bhacca, *Proc. Japan Acad.* **40**, 236 (1964); M. Miyamoto, K. Morita, Y. Kawamatsu, M. Sasai, A. Nohara, K. Tanaka, S. Tatsuoka, K. Nakanishi, Y. Nakadaira and N. S. Bhacca, *Tetrahedron Letters* 2367 (1964).

[26] C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, C. F. Wolf and J. H. Williams, *J. Amer. Chem. Soc.* **74**, 4981 (1952); R. B. Woodward, F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. P. Pasternack, P. N. Gordon, F. J. Pilgrin and K. J. Brunnings, *Ibid.* **75**, 5455 (1953); H. Muxfeldt, *Angew. Chem.* (Int. Ed.) **1**, 372 (1962); H. Muxfeldt and R. Bangert, *Fortschr. Chem. Org. Naturstoffe* **21**, 80 (1963).

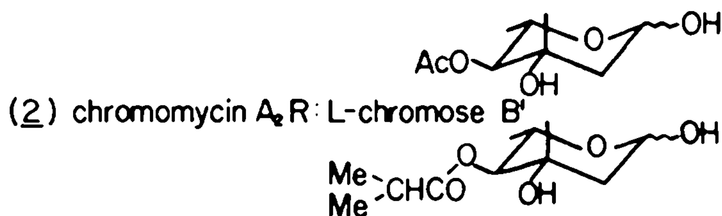
[27] L. Ettlinger, E. Gäumann, R. Hutter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog, P. Reusser and H. Zahner, *Chem. Ber.* **92**, 1867 (1959); H. Brockmann and W. Lenk, *Ibid.* **92**, 1880 (1959); W. D. Ollis, I. O. Sutherland and J. J. Gordon, *Tetrahedron Letters* No. 16, 17 (1959).

[28] S. Marumo, K. Sasaki and S. Suzuki, *J. Amer. Chem. Soc.* **86**, 4507 (1964).

CHART 1



(1) chromomycin A_3 R: L-chromose B

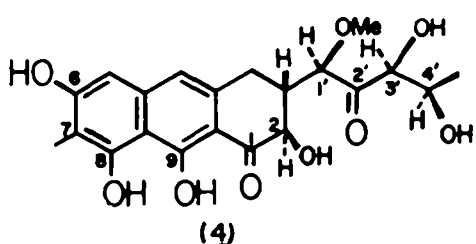


(2) chromomycin A_2 R: L-chromose B'

(3) chromomycin A_4 R: H

(13) monodeacetylchromomycin A_3 R: deacetylchromose B

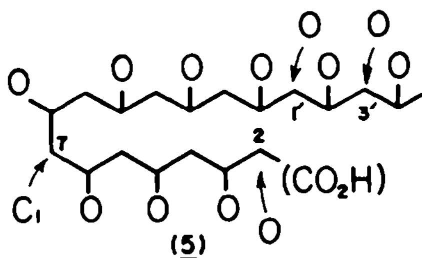
(14) CHR-DCCA ≡ chromomycin A_4 (3) R: H

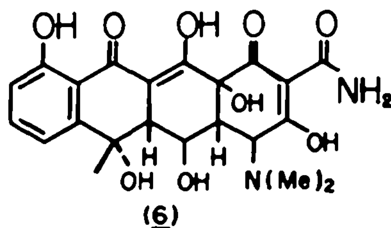


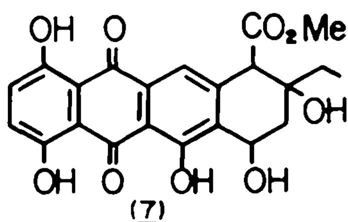
C_2–R
C_3–S
$C_{1'}$–R
$C_{3'}$–S
$C_{4'}$–R

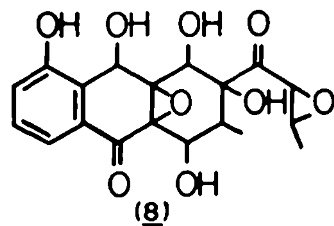
A-D-CHR-C-B
(9)

A-D-CHR-C-C-B
(9a)









olivomycin[19,20] indicate that in this antibiotic too the four sugars corresponding to the chromoses are linked in an identical manner to the olivomycin chromophore. However, from a quantitative determination of the sugars in the olivomycin hydrolysate the Russian workers showed that it contained *two* chromose C units,[19] and, because "the hydrolysate from chromomycin A_3 is indistinguishable from that of olivomycin", stated that the former also contains two moles of chromose C. As described below, a quantitative hydrolysis of chromomycin A_3 hexatosylate shows this to be the case; **9** should therefore be revised to **9a** or **1**.

The number of sugar residues and the manner in which these are attached to the chromophore can be derived unambiguously utilizing only *positive* experimental evidence as described in the sequel. The scheme is summarized in Chart 2.

CHART 2. The sequence of chromoses in chromomycin A_3.

Evidence	Conclusion
(I) Isolation of CHR-8,3′,4′-triacetate.	Sugars can only be linked to C-2 and/or C-6.
(II) Comparison of UV of CHR hexaacetate and CHR–D peracetate.	Chromose D is attached to C-6.
(III) Hydrolysis of monodeacetyl A_3 gives CHR–DCCA.	Chromose B is at terminal.
(IV) Methanolysis of CHR–DCCB hexatosylate affords 4-OTs-chromoside D.	A chromose C–chromose C chain is attached to C-2; chromose B is attached to either one of the chromose C units (see 17).
(V) Methanolysis of A_3 hexatosylate affords 3-OTs-chromoside A and more than one mole of 4-OTs-chromoside C.	Chromose A is at terminal and is attached to chromose D. Entire sequence of A–D–CHR–C–C–B is derived.

(1) C-2 *and* C-6 *of* CHR *are the only two positions for the sugar linkages*

A CHR triacetate **11** was isolated from the 50% formic acid hydrolysate of chromomycin A_3 heptaacetate **10** (since the original antibiotic has already two acetoxyl groups in its sugar part, this is actually a nonaacetate in which all hydroxyls excepting the t-OH in chromose B are acetylated). As reported in our previous paper[14] the NMR chemical shifts of the various acetoxyl peaks attached to CHR fall within a very narrow range characteristic of their positions, i.e., at C-9, C-8, C-6, C-2, C-3′ and C-4′. The chemical shifts of the triacetate **11** are compared with those of CHR

Chemical shifts of acetoxyl and C_7-Me peaks of CHR 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
CHR hexaacetate	2·47	2·41	2·35	2·28	2·23	2·10	2·01
CHR 8,3′,4′-tri-acetate (11)		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.

Chemical shifts of carbinyl protons (ppm)

Position	2	3′	4′
CHR hexaacetate	5·57	5·28	5·42
CHR 8,3′,4′-triacetate (11)	4·28	5·33	5·45

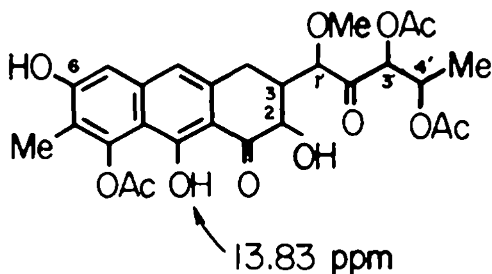
[20] Yu. A. Berlin, S. E. Esipov, M. N. Kolosov and M. M. Shemyakin, *Tetrahedron Letters* 1643 (1966).

CHART 3

A_3 heptaacetate (10)

↓ 50% HCOOH 75-80° 1 hr

CHR triacetate (11)



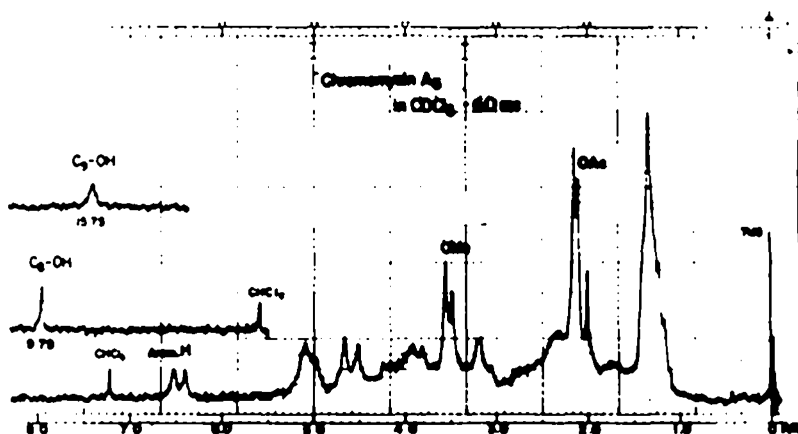


FIG. 1. NMR spectrum of chromomycin A_3 (1)

hexaacetate and the average values in Chart 3, and it is quite clear that the C-9, C-6 and C-2 hydroxyl groups are left unacetylated. The same conclusion is derived by comparing the chemical shifts of the carbinyl protons in the triacetate **11** with those of the hexaacetate (Chart 3); here again the 4·28 ppm signal of the triacetate is located at higher magnetic fields in comparison to the 5·57 ppm C-2 acetoxy carbinyl proton of CHR hexaacetate. These three positions indicate points of attachment, but C-9 can be excluded since the NMR spectrum of chromomycin A_3 itself has two clear peaks at 9·79 and 15·75 ppm, which can, respectively, be assigned to the C-8 and C-9 hydroxyl groups[14] (Fig. 1). Thus the C-2 and C-6 positions are the only two possible positions for the sugars to be linked.

(II) *Chromose* D *is attached to* C-6

The CHR–D fraction obtained from the hydrolysis of chromomycin A_3 with 50% acetic acid yielded a crystalline peracetate **12** upon acetylation, the UV and NMR

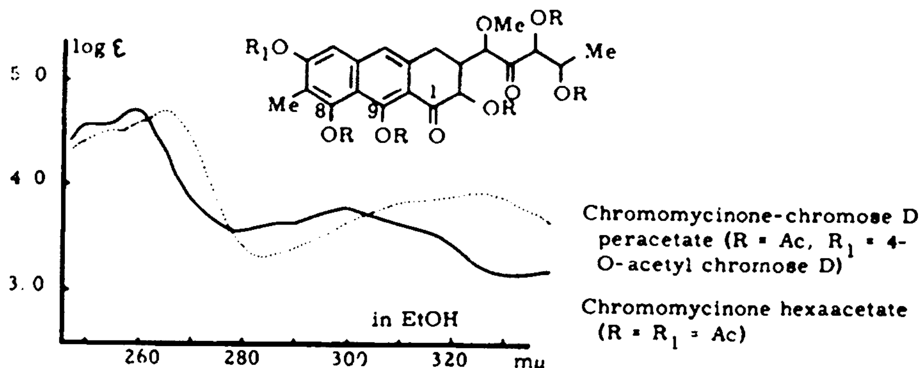


FIG. 2. UV spectra of chromomycinone hexaacetate and chromomycinone-chromose D peracetate (12)

FIG. 3. NMR spectrum of chromomycinone-chromose D peracetate (12)

spectra of which are shown in Figs. 2 and 3. As indicated in Fig. 2, the UV max are located at longer wavelengths as compared to CHR hexaacetate; of the three hydroxyl groups at C-9, C-8 and C-6 in CHR, which could induce a change in the UV spectrum because of the difference in being linked to chromose D and acetyl residues, the C-9 and C-8 hydroxyls are excluded because of the mentioned 9·79 and 15·75 ppm NMR peaks. Therefore, the difference in UV spectra enables one to conclude that chromose D is attached to C-6. Indeed, it is well-known[30] that the UV max of phenolic ethers are located at longer wavelengths than the corresponding acetates, and since the effect of a glycosidic linkage can be regarded as being similar to an ether linkage from a spectroscopic view point, the bathochromic shifts noticed in Fig. 2 can be accounted for by the attachment of chromose D instead of an acetyl group at C-6. Furthermore, in the NMR spectrum of CHR-D peracetate **12** (Fig. 3), no acetoxyl peak is seen in the immediate vicinity of 2·35 ppm, the position characteristic of C-6 acetoxyl groups[14] (see Chart 3); this provides further corroborative evidence for the attachment of chromose D to C-6.

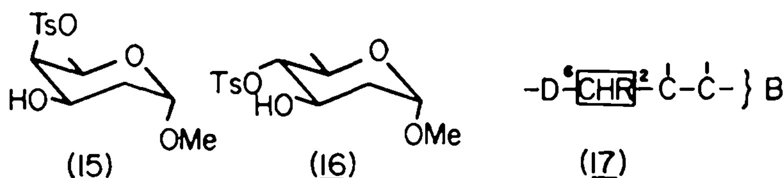
[30] A. I. Scott, *Interpretation of the Ultra-violet Spectra of Natural Products* p. 91. Pergamon Press. London (1964).

(III) *Chromose* B *is at terminal position*

The acetyl group linked to the chromose B residue in chromomycin A_3 (**1**) was found to be selectively hydrolysed upon careful treatment with aqueous methanolic potassium carbonate, thus yielding monodeacetylchromomycin A_3 (**13**, Chart 1). Hydrolysis of **13** with 50% acetic acid now preferentially cleaved the glycoside linkage between chromose C and B instead of that between chromose D and A, and gave the fragment CHR-DCCA (**14**, Chart 1; see discussion on structure of chromomycin A_4). Since chromose B is not contained in this partial hydrolysate, chromose B must be at a terminal position.

(IV) *Chromose* D *is terminal in the* CHR-D-C-C-B *fragment*

It was then found that methanolysis of the hexatosylate[31] prepared from the mentioned CHR-DCCB fragment (all hydroxyl groups excepting C_9-OH and the t-OH in chromose B are tosylated) yielded methyl 4-O-tosyl-3-deacetyl-α-D-chromoside D (**15**), as well as the methyl glycosides of chromose B and 4-O-tosyl-α-D-chromose C [**16**]. The positions of the tosyl groups in the chromosides **15** and **16** were determined by their acid hydrolysis to monotosyldeacetylchromose D and monotosylchromose C,



The numerals 2 and 6 indicate points of attachments of sugar residues.

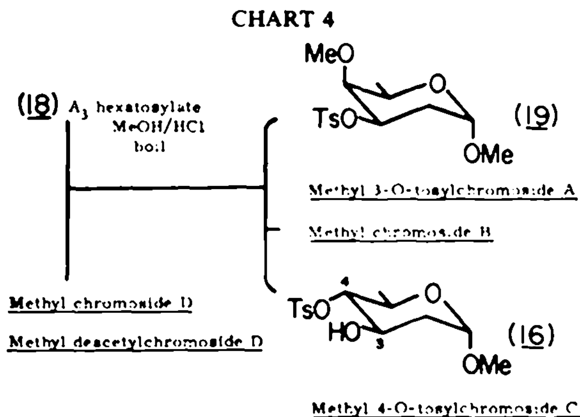
respectively, and the inertness of these free sugars to the action of periodic acid. Production of the glycoside **15** indicates that chromose D should occupy a terminal position in the CHR-DCCB fragment. Moreover, since it has already been shown that chromose B occupies a terminal position in the original antibiotic (see III above), and that chromose D is attached to C-6 of the chromophore (see II above), the two chromose C units should be linked to C-2. Thus partial structure **17** is derived. The presence of two chromose C units rather than one follows from the quantitative experiment described in the next section (V).

(V) *Chromose* A *is at terminal position, and the* C_4-OH *groups in both chromose* C *units are free*

Finally, as shown in Chart 4, methanolysis of chromomycin A_3 hexatosylate (**18**)[31] (all hydroxyl groups excepting the C_9-OH in CHR and the t-OH in chromose B are tosylated) afforded methyl chromoside D and methyl deacetylchromoside D from the water-soluble fraction, and methyl 3-O-tosyl-α-D-chromoside A (**19**), methyl 4-O-tosyl-α-D-chromoside C (**16**) and methyl α-L-chromoside B from the lipid-soluble fraction. Since chromose A has only one hydroxyl group besides the anomeric C_1-OH, chromose A obviously occupies a terminal position.

[31] The number of tosyl groups introduced were estimated from elementary analyses, integrated area of aromatic methyl peak in NMR spectra, and observation of the low-field (15–16 ppm) NMR peak due to the C-9 hydroxyl group of the chromophore.

CHART 4

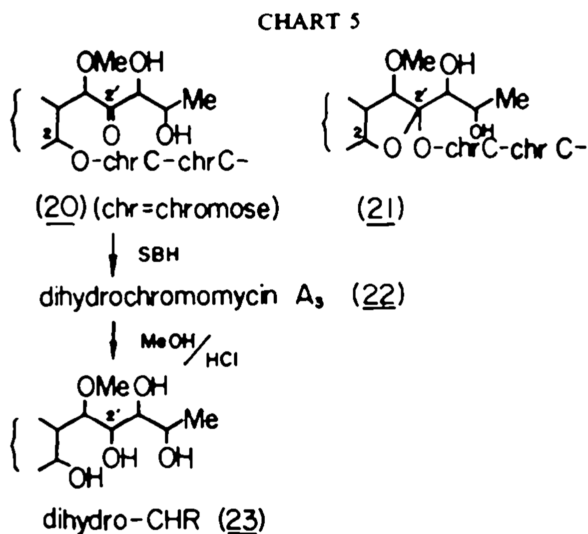


In a quantitative methanolysis starting from 2·1 g of the hexatosylate, and weighing the sugar produced, it was found that 506 mg of the chromose C tosylate **16** was produced; in another run starting from the same amount, 416 mg of tosylate **16** was produced. This corresponds to 1·6 mole and 1·32 mole of the chromose C unit. It is therefore evident that two units of chromose C are present and that the hydroxyl functions at C-4 in the two chromose C units are *both* free in the original antibiotic. Accordingly, it follows that in partial structure **17**, chromose B is attached to C-3 of the *terminal* chromose C.

Finally, the remaining chromose A can only be linked to chromose D, and this completes the elucidation of the linking sequence of the five chromoses.

However, in view of the ease of hemiketal formation between the C-2 hydroxyl and C-2′ carbonyl in CHR derivatives,[14] a further choice had to be made between two possible structures **20** and **21**. Of the two alternative structures, **21** could be eliminated for the following reasons (see Chart 5). Reduction of chromomycin A_3 with sodium borohydride gave dihydrochromomycin A_3 (**22**), which upon hydrolysis with methanol

CHART 5



containing a drop of hydrochloric acid gave dihydro-CHR (23). Since this was identical with the specimen obtained previously[14] by reducing CHR directly with sodium borohydride, the sugar could not have been linked to CHR by a hemiketal linkage as in **21**; the fact that no sugar residue was cleaved in the borohydride reduction step leading to **22** was ascertained by hydrolysing the dihydrochromomycin A_3 and identifying the four sugars. Attempts were also made to detect the side-chain carbonyl group by means of IR spectroscopy but in most cases this was not feasible because of overlap with the acetate bands. However, the IR spectrum (KBr disk) of the CHR–DCCA fragment **14** (Chart 1) clearly showed three absorptions in the carbonyl stretching region at 1738 (acetoxyl of chromose D), 1728 (side-chain ketone) and 1630 cm^{-1} (chelated ketone).

The total structure of chromomycin A_3 is thus represented by expression **1**. This corresponds to a molecular formula of $C_{57}H_{82}O_{26}$.

In an attempt to clarify the nature of the glycoside linkages, the rotatory contribution of each chromose was estimated from the molecular rotation differences between appropriate fragments, and these were compared with the values calculated by Whiffen's method.[32] The values of the acetates were compared because they could be obtained in a pure form more easily than the free compounds. Table 1 shows that there is considerable discrepancy between the observed and calculated sets

TABLE 1. FOUND AND CALCULATED[32] ROTATORY CONTRIBUTIONS OF EACH SUGAR RESIDUE (BASED ON ACETATES)

	Acetate of:	$[M]_D$	Contribution of Sugars	Calculated $[M]_D$ for anomeric methyl glycosides*	Estimation
a.	AD–CHR–CCB′ (A_2)	−661°	B′: a–d = −710°	B′: α − 236° β + 77°	α-B′
b.	AD–CHR–CCB (A_3)	−384°	A: b–c = +656° or d–e = +560°	A: α + 267° β − 46°	α-A
c.	D–CHR–CCB	−1040°	B: b–d = −433° or c–e = −529°	B: α − 236° β + 77°	α-B
d.	AD–CHR–CC (A_4)	+49°			
e.	D–CHR–CC	−511°	CC: e–f = −275°	C: α + 202° β − 111°	β-C/β-C
f.	D–CHR	−236°	D: f–g = −151°	D: α + 267° β − 46°	β-D
g.	CHR	−85°			

* Since parameters for the acyl groups are unknown, the calculated values are those for nonacylated methyl glycosides.

of values. Although the values seem to suggest the following: α-D-chromose A, α-L-chromose B, β-D-chromose C and β-D-chromose D, this result contradicts the configurational uniformity encountered in 6-deoxypyranosides, especially the macrolide antibiotics, i.e., α-L and β-D.[33] The NMR technique was not applicable because of the complex pattern arising from the CHR moiety, and establishment of this final aspect therefore has to be left for the future.

[32] D. H. Whiffen, *Chem. & Ind.* 964 (1956).

[33] W. D. Celmer, *J. Amer. Chem. Soc.* 87, 1799 (1965); see also W. Klyne, *Biochem. J.* 47, xli (1950).

Chromomycin A_2

The spectroscopic properties of chromomycin A_2 are very similar to chromomycin A_3 excepting that slight differences are detected in the NMR spectra (Figs. 1 and 4). Namely, in the spectrum of chromomycin A_2, the integrated area of the methyl peaks around 1·3 ppm is greater, and there is only one acetoxyl peak at 2·1 ppm. This in conjunction with the slight difference in the elementary analyses suggested that one acetoxyl group of chromomycin A_3 was probably replaced by a larger acyl group in A_2. Accordingly, chromomycin A_2 was hydrolysed under conditions that afforded monodeacetylchromomycin A_3 (**13**, Chart 1), and it was found that indeed the same hydrolysis product was formed as characterized by analyses and spectroscopic properties. Furthermore, acetylation of the monodeacetylchromomycin A_3 obtained from A_2 yielded chromomycin A_3 heptaacetate (**10**, i.e., actually a nonaacetate, see above)

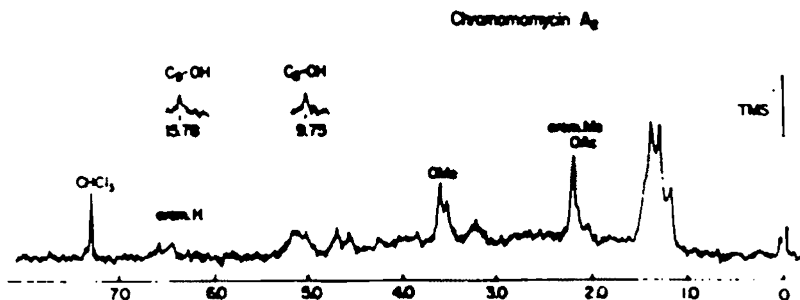


FIG. 4. NMR spectrum of chromomycin A_2 (**2**) in $CDCl_3$, ppm from internal TMS, 60 Mc.

thus confirming that the only difference between A_2 and A_3 was the nature of the ester group attached to chromose B.

Therefore efforts were directed in isolating the chromose B moiety carrying the acyl group intact. This was achieved by treating chromomycin A_2 with 5% methanolic hydrogen chloride[21] upon which it was found that the lipid soluble fraction of the hydrolysate contained a sugar ester in addition to CHR. The NMR spectrum of this sugar, which was tentatively named methyl chromoside B′, showed the presence of an isopropyl group at 1·21 ppm, and moreover, comparison with the methyl chromoside B spectrum suggested that methyl chromoside B′ carried an isobutyryl group instead of the acetyl group (see structure of chromose B′ in structure **2**, Chart 1); alkaline hydrolysis of methyl chromoside B′ gave methyl deacetylchromoside B and isobutyric acid, the latter being identified as its p-bromophenacyl ester. Thus, chromose B′ is 4-O-isobutyryldeacetyl-L-chromose B.[21,22]

Methanolysis of chromomycin A_2 hexatosylate[21] under conditions similar to those employed in the case of the A_3 hexatosylate **18** (see Chart 4) afforded methyl deacetylchromoside D from the water-soluble fraction, and methyl 3-O-tosyl-α-D-chromoside A (**19**), methyl 4-O-tosyl-α-D-chromoside C (**16**) and methyl 4-O-isobutyryldeacetyl-L-chromoside B (mostly α anomer, contaminated with a small amount of the β anomer). The structure of chromomycin A_2 can therefore be represented by **2** (Chart 1), the molecular formula of which is $C_{59}H_{86}O_{26}$.

Chromomycin A_4

The spectroscopic, chromatographic, and chemical properties were identical to those of CHR-DCCA **14**, the partial hydrolysis product of chromomycin A_3. Moreover, both chromomycin A_4 and CHR-DCCA gave an identical octaacetate (actually a nonaacetate because chromose D already has one acetoxyl group).

The presence of two chromose C units in chromomycin A_4 was unambiguously demonstrated in the same manner as in the case of chromomycin A_3; a quantitative methanolysis of chromomycin A_4 heptatosylate[21] yielded 0·93 mole of methyl di-O-tosylchromoside C and 0·44 mole of methyl mono-O-tosylchromoside C. Thus the structure of chromomycin A_4 should be represented by **3** in Chart 1, the molecular formula being $C_{48}H_{68}O_{22}$.

EXPERIMENTAL

IR spectra: JASCO DS-301 and Hitachi EPI-S2 models; UV spectra: Hitachi EPS-2 model; NMR spectra: Varian A-60 spectrometer, chemical shifts are expressed in ppm from internal TMS; s, singlet; d, doublet; t, triplet; q, quartet. M.ps are uncorrected.

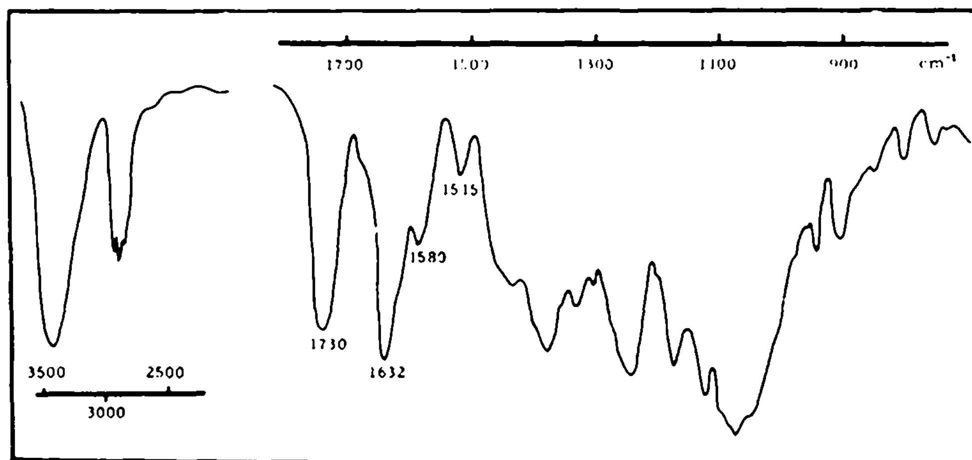


FIG. 5. IR spectrum of chromomycin A_3 in KBr.

Separation of chromomycins A_2, A_3 *and* A_4

The pulverous chromomycin A mixture (10 g) was dissolved in a small amount of AcOEt and added to a column (4 cm × 70 cm) filled with a suspension of silica gel (Merck, 0·05–0·20 mm) in AcOEt containing 1% oxalic acid. The column was eluted with the same AcOEt–oxalic acid system, the eluates being fractionated by checking with TLC. The respective fractions were then concentrated to dryness after washing thoroughly with water.

(i) *First fraction.* Yellow red powder, presumably corresponding to chromomycin A_1 described by Mizuno;[1] however, this was not investigated further because of the very small quantity.

(ii) *Second fraction.* Chromomycin A_2 (**2**), 1·1 g of yellow powder, $[\alpha]_D^{20}$ = −61° (c = 1·0 in EtOH). (Found: C, 58·15; H, 7·21; OMe, 4·92. $C_{59}H_{86}O_{26}$ requires: C, 58·50; H, 7·16; OMe, 5·11%.) λ_{max}^{EtOH} (log ε) 229 mμ (4·22), 279 (4·71), 317 (3·90), 331 (3·79), 412 (3·93). ν_{max}^{KBr} 3400, 1730, 1715 (shoulder), 1630, 1205, 1065 cm⁻¹. NMR, see Fig. 4.

(iii) *Third fraction.* Chromomycin A_3 (**1**), 7 g of yellow powder, $[\alpha]_D^{20}$ = −57° (c = 1·0 in EtOH). (Found: C, 57·48; H, 7·09; OMe, 5·18. $C_{57}H_{82}O_{26}$ requires: C, 57·86; H, 6·99; OMe, 5·24%.) λ_{max}^{EtOH} (log ε) 230 mμ (4·39), 281 (4·72), 304 (3·85), 318 (3·92), 330 (3·84), 412 (4·07). IR, see Fig. 5. NMR, see Fig. 1.

(iv) *Last fraction.* Chromomycin A_4 (3), 0·7 g, $[\alpha]_D^{21} = -47°$ (c = 1·0 in EtOH). (Found: C, 58·00; H, 6·83; OMe, 6·06. $C_{48}H_{68}O_{22}$ requires: C, 57·82; H, 6·88; OMe, 6·22%.) λ_{max}^{EtOH} (log ε) 230 mμ (4·27), 279 (4·67), 318 (3·85), 332 (3·77), 415 (3·93). ν_{max}^{KBr} 3430, 2950, 1740, 1730 (shoulder), 1633, 1580, 1510, 1375, 1240, 1075 cm^{-1}.

Acetates of chromomycin A_2, A_3, *and* A_4

Chromomycins were acetylated with pyridine and Ac_2O by leaving the sol for 1 week at room temp; the products were worked up in the usual manner and recrystallized from EtOH.

(i) *Chromomycin* A_2 *heptaacetate.* M.p. 223°. $[\alpha]_D^{23} = -43·9°$ (c = 1·0 in $CHCl_3$). (Found: C, 58·27; H, 6·57; OMe, 4·40. $C_{73}H_{100}O_{33}$ requires: C, 58·23; H, 6·69; OMe, 4·12%.) λ_{max}^{EtOH} (log ε) 267 mμ (4·81), 315 (4·00), 328 (4·03). ν_{max}^{chf} 3500, 1740, 1625, 1370, 1240, 1080, 1045 cm^{-1}.

(ii) *Chromomycin* A_3 *heptaacetate* (10). M.p. 214°. $[\alpha]_D^{23} = -20°$ (c = 1·0 in EtOH), $[\alpha]_D^{23} = -26°$ (c = 1·0 in $CHCl_3$). (Found: C, 57·53; H, 6·66; OMe, 4·33. $C_{71}H_{96}O_{33}$ requires: C, 57·71; H, 6·55; OMe, 4·20%.) λ_{max}^{EtOH} (log ε) 267 mμ (4·82), 329 (4·05). ν_{max}^{chf} 3500, 3000, 1740, 1692, 1630, 1210, 1045 cm^{-1}.

(iii) *Chromomycin* A_4 *octaacetate.* M.p. 189–191°. $[\alpha]_D^{25} = +3·7°$ (c = 1·0 in $CHCl_3$). (Found: C, 57·68; H, 6·40; OMe, 4·53. $C_{64}H_{84}O_{30}$ requires: C, 57·65; H, 6·35; OMe, 4·65%.) λ_{max}^{EtOH} (log ε) 224 mμ (4·47), 266 (4·79), 318 (3·98), 328 (4·02). ν_{max}^{chf} 1740, 1700, 1635, 1450, 1370, 1050 cm^{-1}. NMR ($CDCl_3$) δ 1·25 (15H, broad), 1·88 (3H, s, OAc), 1·95 (3H, s, OAc), 1·98 (3H, s, OAc), 2·01 (6H, s, OAc), 2·08 (3H, s, OAc), 2·12 (3H, s, arom Me), 2·14 (3H, s, OAc), 2·32 (3H, s, OAc), 2·40 (3H, s, OAc), 3·27 (3H, s, OMe), 3·42 (3H, s, OMe), 3·80, 4·50, 5·10 (all broad), 7·00 (1H, s, arom H), 7·30 (1H, s, arom H).

Acid hydrolysis of chromomycin A_3

Chromomycin A_3 (15 g) was dissolved in 50% AcOHaq and was allowed to stand at 65° for 2 hr. Then water was added to the reaction mixture and extracted with AcOEt. The extract washed with water, dried and evaporated *in vacuo*. The residue showed four spots on TLC (silica gel treated with 1% oxalic acid, developed with AcOEt). They corresponded to CHR, CHR-D, CHR-DCC and CHR-DCCB, respectively. The residue (10 g) was dissolved in AcOEt and chromatographed on a column packed with silica gel treated with 1% oxalic acid (600 g), and eluted with AcOEt containing 1% oxalic acid. The fraction showing one spot on the TLC was collected and evaporated under red press.

The first fraction yielded a solid (1 g) which was identical with chromomycinone (4).

The second fraction yielded a solid (0·9 g) which corresponded to CHR-D. $[\alpha]_D^{21} = -35°$ (c = 1·0 in EtOH), $[\alpha]_D^{21} = -37°$ (c = 1·0 in $CHCl_3$). (Found: C, 58·60; H, 6·28; OMe, 5·58. $C_{29}H_{36}O_{13}$ requires: C, 58·78; H, 6·12; OMe, 5·23%.) λ_{max}^{EtOH} (log ε) 229 mμ (4·44), 277 (4·67), 316 (3·84), 328 (3·71), 410 (3·89). ν_{max}^{KBr} 3400, 1725, 1630, 1580, 1090, 1040 cm^{-1}.

The third fraction yielded a solid (2 g) which corresponded to CHR-DCCB, $[\alpha]_D^{21} = -79°$ (c = 1·0 in EtOH). $[\alpha]_D^{21} = -69°$ (c = 1·0 in $CHCl_3$). (Found: C, 57·84; H, 6·86. $C_{50}H_{70}O_{23}$ requires: C, 57·80; H, 6·79%.) λ_{max}^{EtOH} (log ε) 229 mμ (4·37), 279 (4·70), 317 (4·31), 331 (4·21), 412 (4·38). ν_{max}^{KBr} 3450, 1730, 1630, 1370, 1240, 1070 cm^{-1}.

The fourth fraction yielded a solid (3 g) which corresponded to CHR-DCC. $[\alpha]_D^{24} = -101°$ (c = 1·0 in EtOH). (Found: C, 57·79; H, 6·62. $C_{41}H_{56}O_{19}$ requires: C, 57·74; H, 6·62%.) λ_{max}^{EtOH} (log ε) 229 mμ (4·47), 278 (4·76), 317 (3·97), 330 (3·87), 412 (4·02). ν_{max}^{KBr} 3450, 1725, 1632, 1400, 1060 cm^{-1}.

Acetates of hydrolysis products

The hydrolysis products obtained from chromomycin A_3 were acetylated with pyridine–Ac_2O. The reaction mixture was allowed to stand at room temp for 3 days, and poured into ice-water. The ppts were collected, dissolved in chf and chromatographed on silica gel treated with 1% oxalic acid. The column was eluted with a 100:10:1 mixture of chf–acetone–EtOH, to give the following acetates as the main product in the respective cases.

(i) CHR-D *peracetate* (12). Recrystallization from EtOH yielded needles, m.p. 200°, $[\alpha]_D^{23} = -35°$ (c = 1·0 in EtOH), $[\alpha]_D^{23} = -28°$ (c = 1·0 in chf). (Found: C, 58·11; H, 5·74; OMe, 3·83. $C_{41}H_{48}O_{19}$ requires: C, 58·29; H, 5·73; OMe, 3·68%.) UV, see Fig. 2. ν_{max}^{chf} 1745, 1698, 1630, 1370, 1230 cm^{-1}. NMR, see Fig. 3.

(ii) CHR-DCCB *heptaacetate*. $[\alpha]_D^{24} = -78°$ (c = 1·0 in chf). (Found: C, 57·29; H, 6·42. $C_{64}H_{84}O_{30}$ requires: C, 57·65; H, 6·35%.) λ_{max}^{EtOH} (log ε) 223 mμ (4·46), 265 (4·80), 312 (shoulder), 324 (4·03). ν_{max}^{chf} 3500, 1745, 1695, 1630, 1370, 1240, 1045 cm^{-1}.

(iii) CHR-DCC *octaacetate*. $[\alpha]_D^{24} = -43°$ (c = 1·0 in chf). (Found: C, 57·31; H, 6·02. $C_{57}H_{72}O_{27}$ requires: C, 57·57; H, 6·10%.) λ_{max}^{EtOH} (log ε) 224 mμ (4·47), 266 (4·82), 315 (shoulder), 327 (4·01). ν_{max}^{chf} 1745, 1695, 1630, 1370, 1240, 1045 cm^{-1}.

Molecular weight estimations of chromomycins and hydrolysis products

Satisfactory mol. wt determinations could not be carried out by the conventional osmometric method, X-ray analysis[1] and ultra-centrifugal method.[1] The best method was by UV in which the ε of the crystalline CHR-D peracetate, m.p. 200° (Fig. 2), was taken as reference: ε 66,000 at 266 mμ in EtOH.

Acetates of	$E_{1cm}^{1\%}$	MW(found)	MW(calc.)
A_2 (1)	444	1486	1477
A_3 (2)	425	1553	1506
A_4 (3)	465	1419	1333
CHR-DCCB	469	1407	1333
CHR-DCC	551	1198	1189

Chromomycinone triacetate (11). A soln of 3 g of chromomycin A_3 heptaacetate in 300 ml 50% formic acid was heated at 75–80° on the water-bath for 1 hr, and then poured into 1 l. water. This was extracted with AcOEt, the extract was washed with water, $NaHCO_3$aq, and three more times with water, dried over Na_2SO_4, and concentrated, upon which 1·3 g crude powder was obtained. The crude material was dissolved in chf, chromatographed on silica gel containing 1% oxalic acid and eluted with chf to afford a yellow powder. (Found: C, 57·16; H, 5·44. $C_{27}H_{30}O_{12}\cdot H_2O$ requires: C, 57·44; H, 5·71%.) λ_{max}^{EtOH} (log ε) 228 mμ (4·39), 227 (4·72), 334 (3·96), 386 (3·85). ν_{max}^{chf} 3350 (broad), 1735, 1613 cm^{-1}. NMR ($CDCl_3$) δ 1·30 (3H, d, J 6·5, Me), 1·97 (3H, s, OAc), 2·05 (3H, s, arom Me), 2·21 (3H, s, OAc), 2·41 (3H, s, OAc), 3·46 (3H, s, OMe), 4·28 (1H, broad), 5·33 (1H, broad), 5·45 (1H, broad), 6·43 (1H, s, arom H), 6·53 (1H, s, arom H), 13·83 (1H, s, OH).

Monodeacetylchromomycin A_3 (13). To a soln of 8 g of chromomycin A_3 in 80 ml MeOH, a soln of 2 g K_2CO_3 in 60 ml water was added slowly, and the mixture left at room temp for 20 min with occasional stirring. It was then neutralized with 2 g oxalic acid, extracted with AcOEt, and the extract washed with water, concentrated, and chromatographed on silica gel containing 1% oxalic acid. The column was eluted with AcOEt containing 1% oxalic acid to afford 5·5 g of 13, $[\alpha]_D^{23} = -48·6°$ (c = 1·0 in EtOH). (Found: C, 57·97; H, 7·43; OMe, 5·47. $C_{55}H_{80}O_{25}$ requires: C, 57·88; H, 7·07; OMe, 5·43%.) λ_{max}^{EtOH} (log ε) 229 mμ (4·43), 279 (4·67), 318 (3·91), 330 (shoulder), 415 (3·98). ν_{max}^{KBr} 3450, 1740, 1635, 1370, 1233, 1065 cm^{-1}.

Chromomycinone–DCCA (14). The compound 13 (8·5 g) obtained above was dissolved in 170 ml 50% AcOH and heated for 1·5 hr at 50°. The soln was concentrated *in vacuo* at a temp below 50°, the residue was extracted with AcOEt, and the extract washed with water and chromatographed on silica gel containing 1% oxalic acid. AcOEt–1% oxalic acid was employed as eluant. The first fraction contained CHR–DCC, and the second fraction contained the desired CHR–DCCA 14, 3 g; a portion was hydrolysed with 50% AcOH to give the chromoses A, C and D, $[\alpha]_D^{22} = -43°$ (c = 1·0 in EtOH). Analyses, IR and UV spectra were identical with chromomycin A_4.

CHR–DCCA *octaacetate*. CHR–DCCA 14 were acetylated with pyridine and Ac_2O according to the usual procedure, and the crude acetate was chromatographed on silica gel containing 1% oxalic acid. The column was eluted with a 100:10:1 mixture of chf–acetone–EtOH, to give CHR–DCCA octaacetate as the main product. Analyses, m.p., and spectroscopic properties were identical with chromomycin A_4 octaacetate.

CHR-DCCB *hexatosylate*.[21] *p*-Toluenesulfonyl chloride (3 parts) was added in small portions to a soln of CHR-DCCB (1 part) in pyridine (10 parts) while cooling with ice-water. The reaction mixture was kept in the refrigerator for 3 days, and poured into ice-water. The solid was collected, dried, dissolved in chf and chromatographed on silica gel treated with 1% oxalic acid. The chromatogram was developed first with chf and then with a mixture of chf and acetone. The main fractions were combined and evaporated to dryness to yield the hexatosylate. $[\alpha]_D^{22} = -34·5°$

(c = 1·0 in chf). (Found: S, 9·85. $C_{[illegible]}H_{10[illegible]}O_{[illegible]}S_6$ requires: S, 9·78%.) λ_{max}^{EtOH} (log ε) 226 mμ (4·87), 271 (4·57), 317 (3·82), 329 (3·82), 384 (3·52). ν_{max}^{chf} 3500, 1750, 1630, 1600, 1380, 1180 cm^{-1}.

Chromomycin A_3 *hexatosylate* (18). This was prepared and purified in the manner described above. $[\alpha]_D^{21}$ = +11·3° (c = 1·0 in chf). (Found: S, 9·11. $C_{99}H_{118}O_{38}S_6$ requires: S, 9·11%.) λ_{max}^{EtOH} (log ε) 227 mμ (4·95), 272 (4·58), 318 (3·86), 331 (3·86), 386 (3·50). ν_{max}^{chf} 3450, 1745, 1701, 1630, 1380, 1180 cm^{-1}.

General procedure of methanolysis of tosylates

A soln of 1 part of the tosylate in 5–10 parts of THF was treated with 50 parts of 10% MeOH–HCl and boiled on the bath for 12 hr. The soln was concentrated after removal of HCl by passage through a column of IR-45, and the residue was dissolved in benzene. The benzene soln was washed repeatedly with water, and the aqueous layers were combined, hydrolysed with 10% AcOH and used for detection of the respective chromoses by PPC.

The benzene layer was decolourized with active charcoal, and concentrated. A portion of the residue was used for the identification of sugars by applying it to TLC with silica gel containing 1% oxalic acid, drying the TLC plates in an electric oven, and characterizing by means of the colour of spots and R_f values; a 1:19 mixture of AcOEt–chf was used for developing the chromatography. The sugar tosylates appeared in the following order of decreasing R_f values on TLC plates: methyl 3,4-di-O-tosyl-chromoside C (brown spot), methyl 3-O-tosylchromoside A (black brown spot), methyl 4-O-tosylchromoside C (brown spot) and methyl 4-O-tosyldeacetylchromoside D (blue spot).

The bulk of the benzene extract residue was dissolved in chf and fractionated by chromatography on a 1 cm × 70 cm column of silica gel (Merck, 0·05–0·20 mm), using the 19/1 mixture of chf–AcOEt. The separated tosylates were then hydrolysed according to the conditions described below, and the respective chromoses were identified by PPC;[21] methyl 3,4-di-O-tosyl-α-D-chromoside C, methyl 3-O-tosyl-α-D-chromoside A and methyl α-L-chromoside B were compared with authentic samples (see below).

Hydrolysis. A soln of tosylate (50–100 mg) dissolved in excess dioxan was treated with 1 ml 10% NaOH and boiled for 7 hr. The dioxan was removed by evaporation, and the residue was treated with K_2CO_3, and extracted with ether. The ether extract was evaporated and the residual oil was hydrolysed further with 10% AcOH.

Methanolysis of CHR–DCCB *hexatosylate*

The following were obtained by the methanolysis of 2 g of the hexatosylate.

(i) *Methyl 4-O-tosyl-α-D-chromoside* C (16). M.p. 126°; needles (from MeOH), yield 185 mg. $[\alpha]_D^{25}$ = +112° (c = 1·0 in chf). (Found: C, 52·96; H, 6·50; S, 10·40. $C_{14}H_{20}O_6S$ requires: C, 53·14; H, 6·37; S, 10·13%.) λ_{max}^{EtOH} (log ε) 226 mμ (4·12), 263 (2·74), 274 (2·65). ν_{max}^{chf} 3500, 2950, 1600, 1360, 1172, 1130, 1035, 980, 880 cm^{-1}. NMR ($CDCl_3$) δ 1·25 (3H, d, J 6·5), 2·50 (3H, s, arom Me), 3·30 (3H, s, OMe), 4·77 (1H, q, J 10 and 3·5), 7·43 (2H, d, J 8·5, arom H), 7·93 (2H, d, J 8·5, arom H).

(ii) *Methyl 4-O-tosyl-deacetyl-α-D-chromoside* D (15). M.p. 90–91°, needles (from benzene–n-hexane) yield 100 mg. $[\alpha]_D^{25}$ = +143° (c = 0·65 in chf). (Found: C, 53·37; H, 6·38; S, 10·04. $C_{14}H_{20}O_6S$ requires: C, 53·15; H, 6·37; S, 10·14%.) ν_{max}^{chf} 3550, 2950, 1600, 1360, 1175, 1050, 990, 925, 850 cm^{-1}. NMR ($CDCl_3$) δ 1·25 (3H, d, J 6·5), 2·44 (3H, s, arom Me), 3·25 (3H, s, OMe), 4·73 (1H, broad), 7·35 (2H, d, J 8·5, arom H), 7·83 (2H, d, J 8·5, arom H).

(iii) *Methyl deacetylchromoside* B. This was contained in the aqueous layer.

Methanolysis of chromomycin A_3 *hexatosylate* (18)

Methanolysis of 2·1 g (1 mmole) of the hexatosylate 18 afforded the following products.

(i) *Methyl 3-O-tosyl-α-D-chromoside* A (19). M.p. 96° (dec), granules (from MeOH), yield 210 mg (0·64 mmole); a second hydrolysis under identical conditions gave 167 mg (0·50 mmole). $[\alpha]_D^{25}$ = +150° (c = 0·62 in chf). (Found: C, 54·61; H, 6·69; S, 9·59; OMe, 19·01. $C_{15}H_{22}O_6S$ requires: C, 54·53; H, 6·71; S, 9·65; OMe, 18·79%.) λ_{max}^{EtOH} (log ε) 226 mμ (4·07), 262 (2·76). ν_{max}^{chf} 2950, 1600, 1360, 1175, 1050, 935, 850 cm^{-1}. NMR ($CDCl_3$) δ 1·23 (3H, d, J 6·5), 2·48 (3H, s, arom Me), 3·28 (3H, s, OMe), 3·61 (3H, s, OMe), 4·80 (2H, broad), 7·41 (2H, d, J 8·5, arom H), 7·86 (2H, d, J 8·5, arom H).

(ii) *Methyl 4-O-tosyl-α-D-chromoside* C (16). M.p. 126°; needles (from MeOH), yield 506 mg (1·6 mmoles); a second hydrolysis under identical conditions gave 416 mg (1·32 mmoles).

(iii) *Methyl α-L-chromoside* B. Oil 45 mg; IR spectrum was identical with an authentic sample (see below); the hydrolysis product was also identical with deacetylchromose B, as checked by PPC and TLC.

(iv) *Deacetylchromose* D *and chromose* D. These two sugars were contained in the hydrolysate of the aqueous extract.

Derivation of methyl 3-O-tosyl-α-D-chromoside A (19)

To a soln of methyl α-D-chromoside A (200 mg)[21] in 1·4 ml pyridine, *p*-toluenesulfonyl chloride (240 mg) was added under cooling with ice-water. The reaction mixture was allowed to stand at room temp for 15–17 hr, and poured into ice-water. The ppts (300 mg) were collected and recrystallized from MeOH to afford needles, m.p. 95°. $[\alpha]_D^{23}$ = +156° (*c* = 1·1 in chf). (Found: C, 54·52; H, 6·61; S, 9·42; OMe, 19·64. $C_{15}H_{22}O_6S$ requires: C, 54·53; H, 6·71; S, 9·65; OMe, 18·79%.)

Acetylation of methyl deacetylchromoside B

Acetylation of methyl deacetylchromoside B[21] with pyridine and Ac_2O and working up in the usual manner gave methyl α-L-chromoside B, b.p.$_{10}$ 137°, the IR and NMR spectra of which were identical with those of the natural product. (Found: C, 55·02; H, 8·66; Ac, 18·02; OMe, 15·22. $C_{10}H_{18}O_5$ requires: C, 55·04; H, 8·25; Ac, 17·72; OMe, 15·03%.) ν_{max}^{liq} 3450, 1740, 1370, 1240, 1050 cm^{-1}. NMR ($CDCl_3$) δ 1·18 (3H, d, J 6·5), 1·36 (3H, s, Me), 2·13 (3H, s, OAc), 2·70 (1H, s, OH), 3·33 (3H, s, OMe), 4·63 (1H, d, J 10), 4·73 (1H, t J 2·5).

Periodic acid consumption of chromose C *and deacetylchromose* D *monotosylates*

(i) 4-O-*Tosylchromose* C. Methyl 4-O-tosyl-α-D-chromoside C (16) (100 mg) was dissolved in dioxan, treated with 0·1 ml conc. HCl, heated for 2 hr on the bath, and concentrated after removal of HCl with IR-45; this gave 50 mg of an oil which showed a single spot on TLC. $[\alpha]_D^{22}$ = +52·3° (*c* = 1·0 in chf). ν_{max}^{chf} 3500, 2950, 1600, 1360, 1175, 990, 885 cm^{-1}. NMR ($CDCl_3$) δ 1·10 (3H, d, J 6·5, Me), 2·46 (3H, s, arom Me), 3·98 (2H, s, OH), 7·35 (2H, d, J 8·5, arom H), 7·86 (2H, d, J 8·5, arom H).

The amount of periodic acid consumption was measured with a soln of 4-O-tosylchromose C dissolved in 50% MeOH, according to the method described in Ref. 21; no acid was consumed after three days.

(ii) 4-O-*Tosyldeacetylchromose* D. To a soln of methyl 4-O-tosyldeacetylchromoside D (316 mg) in dioxan (1·5 ml), 2N HCl (0·5 ml) was added and the soln heated at 80° for 30 min. After cooling, the soln was diluted with MeOH, deacidified with IR-45, and purified by chromatography on silica gel. Elution with chf–AcOEt (9:1) yielded 60 mg of an oil, single spot on TLC, which was 4-O-tosyldeacetylchromose D. NMR ($CDCl_3$) δ 1·24 (3H, d, J 6·0), 2·48 (3H, s, arom Me), 2·83 (2H, s, OH), 7·38 (2H, d, J 8·5, arom H), 7·85 (2H, d, J 8·5, arom H).

Similar to the case of 4-O-tosylchromose C, the periodic acid consumption of 4-O-tosyldeacetylchromose D was also zero.

Dihydrochromomycin A_3 (22)

To a soln of 1 g chromomycin A_3 in 30 ml EtOH and 1 ml AcOH, 0·4 g $NaBH_4$ was added dropwise under ice-cooling. After leaving the mixture for 1 hr, the excess reagent was decomposed by addition of 1 g oxalic acid. Extraction with AcOEt, and evaporation of solvent from the extract gave 650 mg of a residue which had an R_f value smaller than that of chromomycin A_3 on TLC. Hydrolysis of this residue yielded chromoses A, B, C, D and deacetylchromose D (identified by PPC). (Found: C, 57·94; H, 7·29; OMe, 5·31. $C_{57}H_{84}O_{26}$ requires: C, 57·77; H, 7·14; OMe, 5·23%.) λ_{max}^{EtOH} (log ε) 230 mμ (4·45), 279 (4·63), 317 (3·86), 320 (shoulder), 415 (3·89). ν_{max}^{KBr} 3400, 1741, 1634 cm^{-1}.

Dihydrochromomycinone (23)

Dihydrochromomycin A_3 (0·51 g) was dissolved in MeOH (3 ml) by warming, and after addition of 0·5 ml conc. HCl the soln was maintained at 50° on the water-bath for 15 min. Examination of the reaction mixture by TLC showed that the reaction was complete. The mixture was poured into a large quantity of ice-water, and the aqueous mixture was extracted with a large amount of AcOEt.

The extract was concentrated to give a suspension, which was chromatographed on silica gel containing 1% oxalic acid. The column was eluted with AcOEt–1% oxalic acid, and the eluates containing the main component were combined and concentrated, upon which pulverous ppts were obtained. The ppts (47 mg) were collected and recrystallized from MeOH to afford plates, which were identical with the $NaBH_4$ reduction product[14] of chromomycinone as checked by IR, TLC and m.p.

Deisobutyrylchromomycin A_3 *or monodeacetyl chromomycin* A_3 (13)

To a soln of 2 (1·5 g) in MeOH (15 ml), 4·5% K_2CO_3aq (10 ml) was added in portions, and the mixture left at room temp for 1 hr and then neutralized with 0·5 g oxalic acid. The reaction mixture was extracted with AcOEt, the extract washed with water, dried, concentrated, and the residue purified as usual by chromatography on silica gel–1% oxalic acid and elution with AcOEt–1% oxalic acid to yield 900 mg of a yellow powder.

Analyses, rotation, and spectroscopic properties were identical with monodeacetylchromomycin A_3 (13) resulting from the partial hydrolysis of chromomycin A_3 (see above).

Acetylation of deisobutyrylchromomycin A_3

Acetylation of deisobutyrylchromomycin A_3 with pyridine and Ac_2O, working up of the reaction mixture, and recrystallization of the product from EtOH afforded needles, m.p. 215°, which were identical in every respect to chromomycin A_3 heptaacetate (10). Data for the present compound are: $[\alpha]_D^{26} = -24 \cdot 1°$ (c = 1·0 in chf); ν_{max}^{chf} 3500, 3000, 1740, 1692, 1630, 1210, 1045 cm^{-1}.

Chromomycin A_3 *hexatosylate*

A soln of 1 g chromomycin A_3 in 10 ml pyridine was treated in portions and under ice-cooling with 2 g *p*-toluenesulfonyl chloride. After dissolution of the reagent, the soln was stored for 3 days in the ice-box, poured into ice-water, and the insoluble matter filtered off. The solid was dried, dissolved in chf and chromatographed on silica gel treated with 1% oxalic acid. The chromatogram was developed with a mixture of chf and acetone, and the main fractions were combined and evaporated to dryness to yield 1 g of the hexatosylate, $[\alpha]_D^{26} = -12 \cdot 9°$ (c = 1·0 in chf). (Found: S, 9·01. $C_{101}H_{122}O_{38}S_6$ requires: S, 9·00%.) λ_{max}^{EtOH} (log ε) 273 mμ (4·64), 317 (3·99), 330 (3·92), 390 (3·71). ν_{max}^{chf} 3500, 2950, 1740, 1700, 1620, 1600, 1370, 1175 cm^{-1}.

Methanolysis of chromomycin A_3 *hexatosylate*

A soln of the hexatosylate (1·8 g) in THF (3 ml) was boiled for 8 hr with 50 ml 5% MeOH–HCl. The reaction mixture was processed according to the general procedure employed in the methanolysis of tosylates (see above), upon which the following products were identified. The lipid-soluble fraction contained methyl 3-O-tosyl-α-D-chromoside A, m.p. 96°, 200 mg; methyl 4-O-tosyl-α-D-chromoside C, m.p. 126°, 250 mg; and methyl 4-O-isobutyryldeacetylchromoside B, oil, 150 mg. The respective glycosides were characterized by IR, NMR and m.p. (excepting the syrupy chromoside B derivative). The water-soluble fraction, after hydrolysis, afforded deacetylchromose D (identified by PPC).

Chromomycin A_4 *heptatosylate*

This was prepared and purified in the manner described for chromomycin A_3 hexatosylate. $[\alpha]_D^{22} = -64 \cdot 5°$ (c = 1·0 in chf). (Found: S, 10·77. $C_{97}H_{110}O_{36}S_7$ requires: C, 10·80%.) λ_{max}^{EtOH} (log ε) 226 mμ (4·96), 274 (4·50), 317 (3·84), 330 (3·80), 386 (3·68). ν_{max}^{chf} 3000, 1750, 1700, 1630, 1600, 1370, 1175 cm^{-1}.

Methanolysis of chromomycin A_4 *heptatosylate*

A soln of the heptatosylate (1·6 g; 0·8 mmole) in THF (10 ml) was boiled for 12 hr with 100 ml 10% MeOH–HCl.

The reaction mixture was processed according to the general procedure employed in the methanolysis of tosylates (see above), upon which the following products were identified.

(i) *Methyl 3,4-di-O-tosyl-α-D-chromoside* C. Oil 351 mg (0·75 mmole). $[\alpha]_D^{25} = +67 \cdot 5°$ (c = 1·0 in chf). (Found: C, 53·60; H, 5·56; S, 14·30. $C_{21}H_{26}O_8S_2$ requires: C, 53·59; H, 5·57; S, 13·62%.)

ν_{max}^{chf} 2800, 1600, 1360, 1180, 1000, 906, 890 cm^{-1}. NMR ($CDCl_3$) δ 1·25 (3H, d, J 6·5), 2·46 (6H, s, arom Me), 3·28 (3H, s, OMe), 4·46 (1H, t, J 9·5), 4·70 (1H, broad s), 7·33, 7·41, 7·70, 7·88 (all 2H, d, J 8·5, arom H).

(ii) *Methyl 4-O-tosyl-α-D-chromoside* C (16). M.p. 126°. 110 mg (0·35 mmole).

(iii) *Methyl 3-O-tosyl-α-D-chromoside* A (19). M.p. 96° (dec), yield 176 mg (0·53 mmole).

(iv) *Methyldeacetyl chromoside* D. This was contained in the aqueous layer.

Methyl 3,4-di-O-tosyl-α-D-chromoside C

To a soln of methyl α-D-chromoside C[21] (170 mg) in pyridine (1 ml), *p*-toluenesulfonyl chloride (500 mg) was added under cooling with ice-water. The reaction mixture was allowed to stand at room temp for 24 hr, and poured into ice-water. The aqueous mixture was extracted with AcOEt, the extract washed with water, dried and concentrated.

The residue was purified as usual by chromatography on silica gel; elution with a 19:1 mixture of chf-AcOEt yielded 300 mg of methyl 3,4-di-O-tosyl-α-D-chromoside C. $[\alpha]_D^{21} = +78.0°$ ($c = 0.99$ in chf). (Found: C, 53·50; H, 5·50; S, 14·00. $C_{21}H_{26}O_8S_2$ requires: C, 53·59; H, 5·57; S, 13·62%.) ν_{max}^{chf} 2800, 1600, 1360, 1180, 1000, 906, 890 cm^{-1}. NMR ($CDCl_3$) δ 1·25 (3H, d, J 6·5), 2·45 (6H, s, arom Me), 3·25 (3H, s, OMe), 4·43 (1H, t, J 9·5), 4·61 (1H, broad, s), 7·25, 7·40, 7·58, 7·75 (all 2H, d, J 8·5 arom H).

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