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STRUCTURES AND PROPERTIES OF THE SUGARS OBTAINED FROM THE CHROMOMYCINS

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Abstract—The water soluble fraction of the acid hydrolysate of chromomycin A_3 when chromatographed on cellulose powder yields four new sugars, chromose A, chromose B, chromose C and chromose D. The structures of these sugars have been elucidated. Interestingly, all four are 2,6-dideoxy sugars, and furthermore, chromose B belongs to the L-series while the other three are D-sugars. The hydrolysis of chromomycin A_2 affords a fifth sugar, which has been identified as 4-O-isobutyryldeacetylchromose B.

HYDROLYSIS of chromomycin A_3[1a,1b] with 50% acetic acid yielded the lipid-soluble aglycone, chromomycinone,[2] and a water-soluble fraction. Chromatography of the water-soluble fraction on cellulose powder and development with cyclohexane and ethyl acetate afforded four new sugars, chromose A,[3] chromose B,[4] chromose C[4] and chromose D.[4] As described below, all four sugars turned out to be 2,6-dideoxy sugars, and furthermore, while chromose B belongs to the L-series, the other three belong to the D-series. Methanolysis of chromomycin A_2,[1b] the minor constituent of the chromomycin group of antibiotics, afforded the methyl glycoside of a further sugar, which has been characterized as 4-O-isobutyryldeacetylchromose B[1b] because of its alkaline hydrolysis to deacetylchromose B and isobutyric acid.

The structures of chromose A and chromose D have since been verified by recent synthesis carried out by J. S. Brimacombe *et al.*[5,6] It is interesting to note that hydrolysis of the Russian antibiotic olivomycin has yielded[7] D-chromose A (olivomose),

[1a] K. Nakazawa, M. Shibata, K. Tanabe, Y. Tokui, A. Miyake, H. Hitomi, M. Miyamoto and M. Imanishi, *Jap. Pat.* 12646 (1960); S. Tatsuoka, A. Miyake and K. Mizuno, *J. Antibiot.* Ser. B 332 (1960).

[1b] M. Miyamoto, Y. Kawamatsu, K. Kawashima, M. Shinohara and K. Nakanishi, *Tetrahedron Letters* 545 (1966).

[3] 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).

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

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

[5] J. S. Brimacombe, D. Portsmouth and M. Stacey, *Chem. and Ind.* 1758 (1964); *J. Chem. Soc.* 5614 (1964).

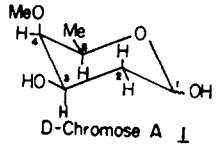
[6] J. S. Brimacombe and D. Portsmouth, *Chem. and Ind.* 468 (1965); *Carbohydrate Research* **1**, 125 (1965).

[7] Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, M. M. Shemyakin and M. G. Brazhnikova, *Tetrahedron Letters* 1323, 3513 (1964).

D-chromose C (olivose), and L-deacetylchromose B (olivomycose); moreover, the last-named sugar is present both in chromomycin A_2[1b] (see below) and in olivomycin in the form of its isobutyrate.

D-*Chromose* A (2,6-*dideoxy*-4-O-*methyl*-D-lyxo-*hexopyranose*)

Chromose A (**1**), $C_7H_{14}O_4$, is the sugar that is isolated first in the stepwise hydrolysis of chromomycin A_3. Chromose A gives a 2,4-dinitrophenylhydrazone $C_{13}H_{16}O_6N_4$. Analysis of the NMR spectrum (Fig. 1) led to two alternative structures **2a** and **2b**,



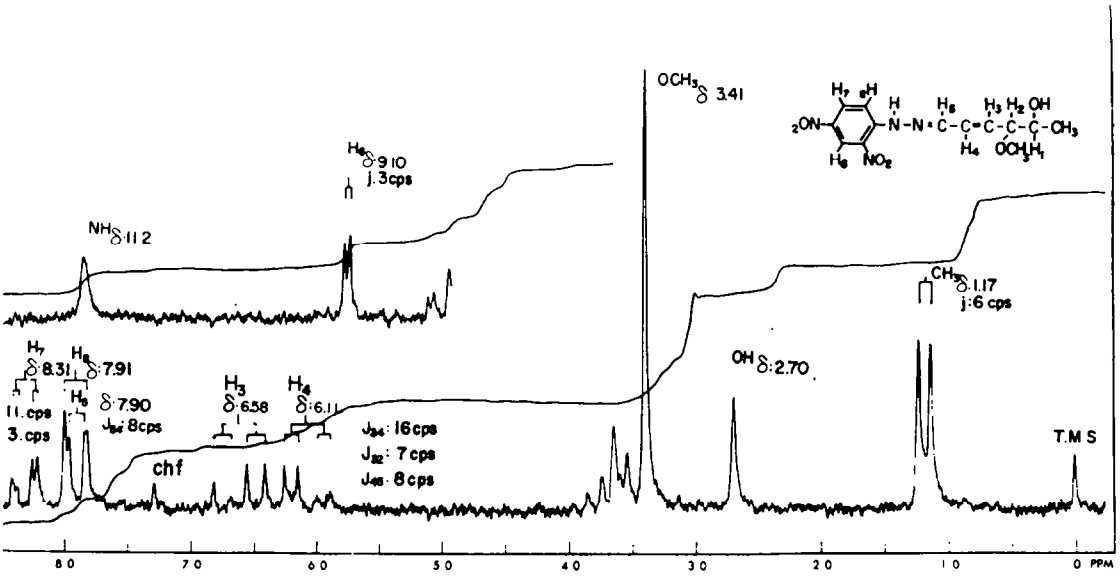
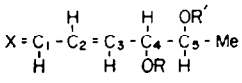


FIG. 1. NMR spectrum of 2,4-dinitrophenylhydrazone (**2a**) in $CDCl_3$, ppm from TMS, 60 Mc.

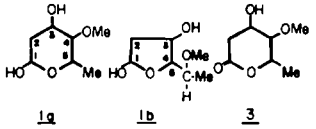


2a R : Me, R' : H, X : 2,4-$(NO_2)_2$-C_6H_3-NH-N

2b R : H , R' : Me, X : 2,4-$(NO_2)_2$-C_6H_3-NH-N

2c R : Me, R' : H, X : O

2d R : H, R' : Me, X : O



1a **1b** **3**

which differ only in the relative positions of the methoxyl and hydroxyl groups. In any case, they are derivatives of α,β-unsaturated aldehydes, but chromose A itself is apparently not the simple aldehyde **2c** or **2d** because of its molecular formula, its transparency in the usual UV region, and absence of IR carbonyl absorption. Accordingly, dehydration must have occurred during the course of formation of the hydrazone, and mechanistic considerations lead to two alternative hemiacetal structures **1a** and **1b**, corresponding respectively to **2a** and **2b**. However, chromose A must be represented by the pyranose structure **1a** since bromine oxidation[8] yielded a six-membered lactone **3**, $\nu_{max}^{liq.}$ 1733 cm^{-1}, exhibiting a positive iodoform reaction after

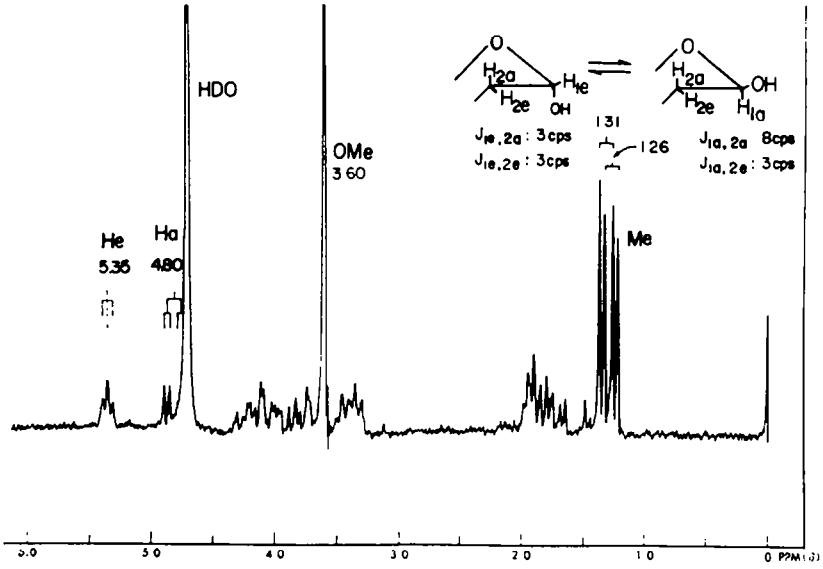


FIG. 2. NMR spectrum of chromose A in D_2O (after 3 days), ppm from DSS, 60 Mc.

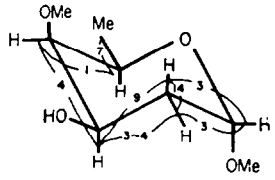
alkaline hydrolysis. The 2,6-dideoxy structure **1a** is fully supported by the NMR spectrum of chromose A (Fig. 2). Thus the two sets of doublets at 1·26 and 1·31 ppm are due to the 6-methyl groups in the anomeric mixture, while the quartet at 4·80 ppm (H_{1a}) and triplet at 5·35 ppm (H_{1e}) indicate that C-2 must be a methylene group. The latter triplet arises from the equatorial proton (H_{1e}) since it is coupled to two adjacent protons, H_{2a} and H_{2e}, with an identical spin-coupling constant of 3 c/s. Although only half of the 4·80 ppm signal is apparent this is evidently assignable to the axial proton H_{1a}, the quartet being the result of axial–axial and axial–equatorial couplings (9 and 3 c/s,[9] respectively) with its neighbors; the quartet is quite clear in the spectrum of the β-chromoside (Fig. 4). The relative intensity of the H_{1a} quartet increased after 2 hr as compared to the intensity of the H_{1e} triplet. Hence the equilibrium is shifted in favor of the equatorial C_1OH, and this shift is responsible for the negative mutarotation (Fig. 2).

Methanolysis of chromomycin A_3 with 5% HCl–MeOH gave two anomeric

[8] S. A. Baker, E. J. Bourne, R. M. Pinkard and D. H. Whiffen, *Chem. and Ind.* 658 (1958).

[9] J. A. Pople, W. G. Schneider and H. J. Bernstein, *High Resolution Nuclear Magnetic Resonance* p. 390. McGraw-Hill, New York (1959).

methyl chromosides **4**, $C_8H_{16}O_4$, and **5** (Fig. 4), which are also obtained from chromose A upon similar acid treatment. The NMR spectrum of one of the anomers **4** (Fig. 3) has a triplet corresponding to H_{1e} at 4·78 arising from spin-coupling to two methylene protons with an identical coupling constant of 3 c/s. On the other hand, the anomer **5** shows a quartet at 4·25 ppm originating from coupling of H_{1a} to adjacent axial and equatorial protons with coupling constants of 9 and 3 c/s, respectively (Fig. 4). As evi-



Methyl α-D-chromoside A (4) (numerals indicate coupling constants).

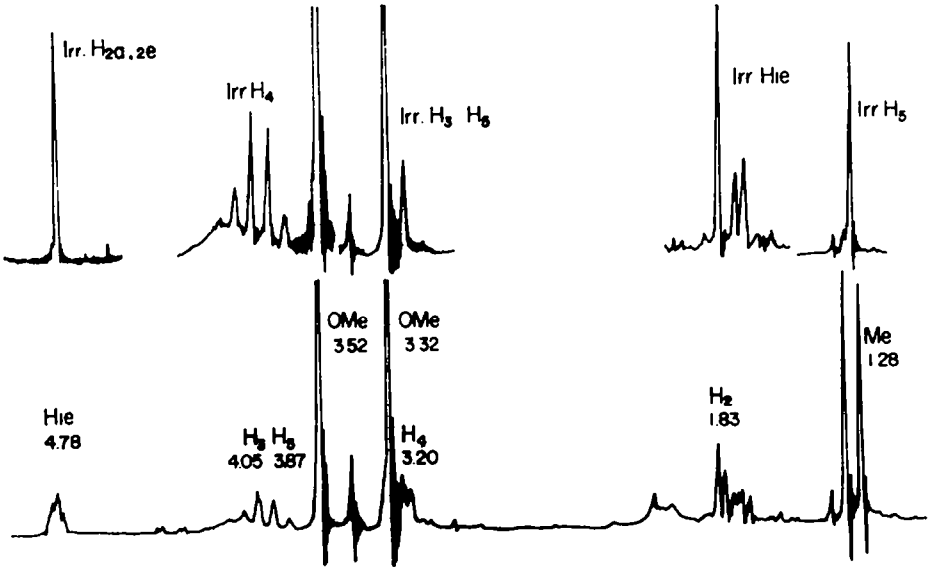


FIG. 3. NMR spectrum of methyl α-D-chromoside A (4) in $CDCl_3$, ppm from TMS, 100 Mc. Decoupled signals are shown in upper trace.

denced from NMR signals due to the anomeric protons, the conformation of anomer **4** is identical with anomer **5**, i.e., the two anomers merely differ in the configuration at C_1. This suggested that there is probably no 1,3-diaxial interaction between substituents in anomer **4** bearing the axial C_1–OMe group, and conformational analyses along this line allowed one to deduce two alternative structures for this anomer, structure **4** or a structure in which only the configuration at C_4 is inverted.

Decoupling experiments shown in Fig. 3 not only showed that C_4–OMe was axial but also fully supported the rest of the structure deduced above. The doublet having a three proton intensity at 1·28* is due to the C_5-secondary methyl group which is coupled to H_5 appearing at 3·87. The complicated two proton multiplet around 1·83 which is due to the C_2-methylene group changes into a pattern similar to the AB part

* In further NMR discussions, the ppm is omitted where no confusion arises.

of an ABK system[10] when the triplet originating from C_1–H_e (at 4·78) is irradiated. This fact indicates that the methylene group is still coupled to one other proton (C_3–H) corresponding to the K part. The triplet nature (J 3 c/s) of the 4·78 signal indicates that H_1 is equatorial.

The one proton signal at 3·87 is a quartet produced by spin–spin coupling with a methyl group and is assigned to C_5–H. Each peak of the quartet is further split by spin-coupling to one adjacent proton at C_4. This is evidenced by the fact that the quartet becomes sharper when the higher field signal due to C_4–H is decoupled. Thus, the coupling constant between C_4–H and C_5–H is found to be 1 c/s from the decoupling

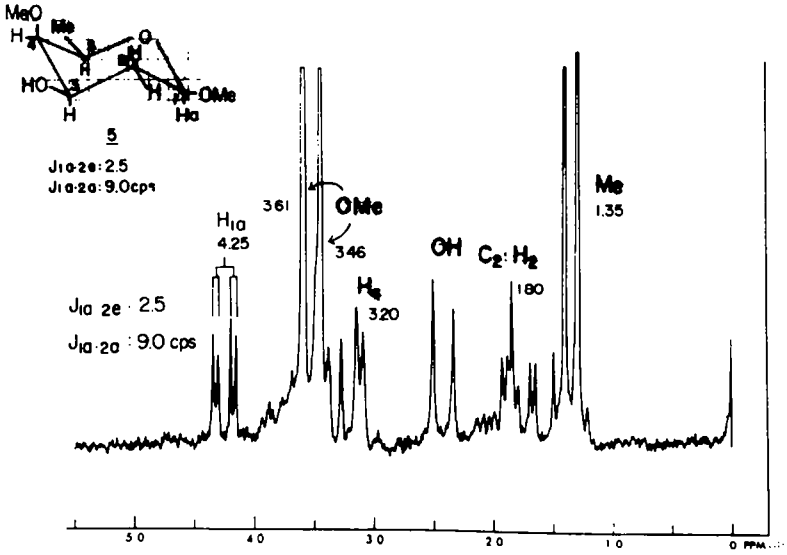


FIG. 4. NMR spectrum of methyl β-D-chromoside A (**5**) in $CDCl_3$, ppm from TMS, 60 Mc.

experiment. This small value corresponds to either an equatorial–equatorial or equatorial–axial coupling constant, and one is lead to the conclusion that C_4–H is equatorial since C_5–Me is shown to be equatorial (C_5-H axial) from the conformational analysis. This is also indicated from the signal due to C_4–H which appears at 3·20 as a doublet of 4 c/s splitting which is further split by 1 c/s. The signal changes into a singlet when a second frequency with an interval of 63 c/s is applied; in this case both C_3–H and C_5–H are decoupled at the same time. As the coupling constant J_{45} is 1 c/s, the constant between C_3–H and C_4–H is 4 c/s, which corresponds to an equatorial–equatorial or equatorial–axial coupling. On the other hand, the hydrogen at C_3 can be found to be axial from the coupling constants of 9 and 3–4 c/s as derived from an analysis of the decoupled C_2-methylene trace. This leads to the conclusion that the hydrogen at C_4 is equatorial. The structure of the chromoside **4**, including the entire set of coupling constants can be derived from Fig. 3, and this in turn leads to structure **1** for chromose A.

The absolute configuration of the sugar can now be derived unambiguously from Hudson's isorotation rule (Fig. 5)[11] and the NMR signals of the anomeric protons.

[10] C. A. Reilly and J. D. Swalen, *J. Chem. Phys.* **32**, 1382 (1960).
[11] C. S. Hudson, *J. Amer. Chem. Soc.* **31**, 66 (1909); **60**, 1537 (1938).

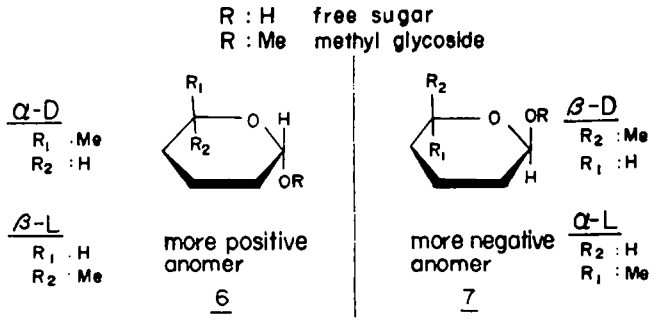


FIG. 5. Hudson's isorotation rule for pyranoses.

As the chromoside **4** is the more positive of the anomers, its absolute configuration at C_1 can be represented by **6**, i.e., **4** is either α-D or β-L. On the other hand, its NMR spectrum and conformational analysis mentioned above requires a *cis* relationship between C_5–Me and C_1–H, and an equatorial orientation for C_1–H. Thus **4** should be a methyl α-D-glycoside: similarly, the more negative anomer **5** is a β-D sugar, and this leads to absolute configurations of the anomeric methyl chromosides **4** and **5** as shown above.

The conclusion that chromose A is a D-sugar is also deduced from the following observations on the free sugar. The direction of mutarotation of chromose A is negative, from +93° to +77°, and this mutarotation corresponds to a shift in equilibrium from **6** to **7** (Fig. 5). On the other hand, in its NMR spectrum measured after 2 hr, the intensity of the signal due to H_{1a} is increased relative to that of H_{1e} as mentioned above (Fig. 2). On the basis of structure **1** from chromose A, a shift from the more positive to the more negative anomer, α-D to β-D or β-L to α-L, would correspond to an anomeric change of H_{1e} to H_{1a} or H_{1a} to H_{1e}, respectively. The actual shift observed is from H_{1e} to H_{1a}, and consequently chromose A must belong to the D-series. The molecular rotations of the chromosides **4** and 5, as calculated according to Whiffen's method,[12] are +267° and −47°, respectively, for the D-series, and this is also in agreement with the observed values of +215° and −63°, respectively.

L-*Chromose* B (2,6-*dideoxy*-3-C-*methyl*-4-O-*acetyl*-L-arabino-*hexopyranose*)

Chromose B (**8**), $C_9H_{16}O_5$, is an oil, $\nu_{max}^{CHCl_3}$ 1733 cm^{-1} (OAc). It does not consume periodate and therefore lacks an α-glycol group. Upon treatment with potassium carbonate, chromose B is hydrolysed to give one mole of acetic acid and deacetylchromose B (**9**), $C_7H_{14}O_4$.

In the NMR spectrum of deacetylchromose B (Fig. 6), the signal due to the anomeric proton can be found at 4·97 as a quartet, which shows the presence of a methylene group on its adjacent carbon atom. The coupling constants obtained from this anomeric signal are 9·5 and 2·8 c/s, which are supported by the double resonance results shown in Fig. 6. They correspond to an axial–axial and an axial–equatorial coupling constant, respectively. These values indicate that this anomeric proton is axial and that deacetylchromose B also has a 2-deoxypyranose structure.

In the spectrum measured after 40 min, a new signal due to an anomeric proton appears as a triplet at 5·33 in addition to the quartet mentioned above. This change

[12] D. H. Whiffen, *Chem. and Ind.* 964 (1956).

is paralleled by mutarotation, and the triplet can be assigned to an equatorial proton located on the anomeric center. In this case, the coupling constants between the anomeric proton and the adjacent methylene are identical, i.e., 3 c/s.

Irradiation of the anomeric axial proton converts the two signals around 1·67 and 2·12 arising from the C_2-methylene group to a pair of doublets with spacings of 12 c/s ($J_{2a,\ 2e}$), which are mirror images of each other, i.e., an AB type quartet. This means

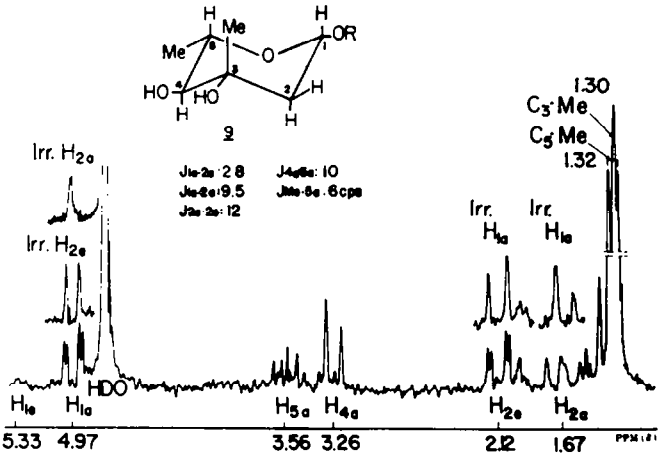
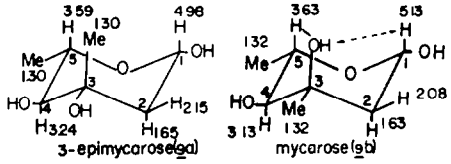


FIG. 6. NMR spectrum and decoupled traces of deacetylchromose B (9) in D_2O, ppm from DSS, 100 Mc. (Small peaks arising from the other anomer are also seen.)



Chemical shifts from the literature[13] have been recalculated to values from internal DSS.

that there is no proton on the adjacent C_3. The resonance pattern of the signal around 3·56 corresponds to that of the proton at C_5. It originates from a spin-coupling to the methyl group with a J of 6 c/s and to the C_4 proton at 3·26 with a J of 10 c/s (axial–axial coupling). This evidence indicates that H_5 and H_4 are both axial and C_5–Me is equatorial. Thus, the structure of deacetylchromose B (9) can be represented by either 9a or 9b.

The NMR spectrum of deacetylchromose B is very similar to the published spectra of mycarose (9b)[13] and 3-epimycarose (9a)[13] which are again similar to each other. They differ only in the configuration at C_3; that is, in 9a, the C_3–OH is equatorial, whereas in 9b, this hydroxyl group is attached as an axial group and is situated in a 1,3-diaxial relation with the C_1 and C_5 protons. Accordingly, the signals due to these two protons are observed at a lower field in 9b than in 9a because of the anisotropy effect of C_3–OH.[14] Comparison of the chemical shifts of H_1 and H_5 signals leads to the

[13] W. Hofheinz, H. Greisebach and H. Friebolin, *Tetrahedron* **18**, 1265 (1962).

[14] Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto and K. Tsuda, *Chem. Pharm. Bull.* **10**, 338 (1962).

conclusion that deacetylchromose B should be represented by 9a or its mirror image.

The direction of mutarotation of deacetylchromose B is negative ($-15° \rightarrow -22°$) and corresponds to the change of either α-D to β-D or β-L to α-L (Fig. 5). As mentioned above, this mutarotation is accompanied by the appearance of an equatorial H_1 signal at 5·33 (Fig. 6). These two observations taken together establish that deacetylchromose B belongs to the L-series.

L-Mycarose has been isolated from magnamycin,[15] foromacidin,[16] spiramycin,[17] tylosin,[18] and others, and the racemic form[19] and L-form[20] have been synthesized. The DL-form of epimycarose has also been synthesized[19] but so far neither the optically active nor inactive forms of epimycarose have been isolated from natural sources.

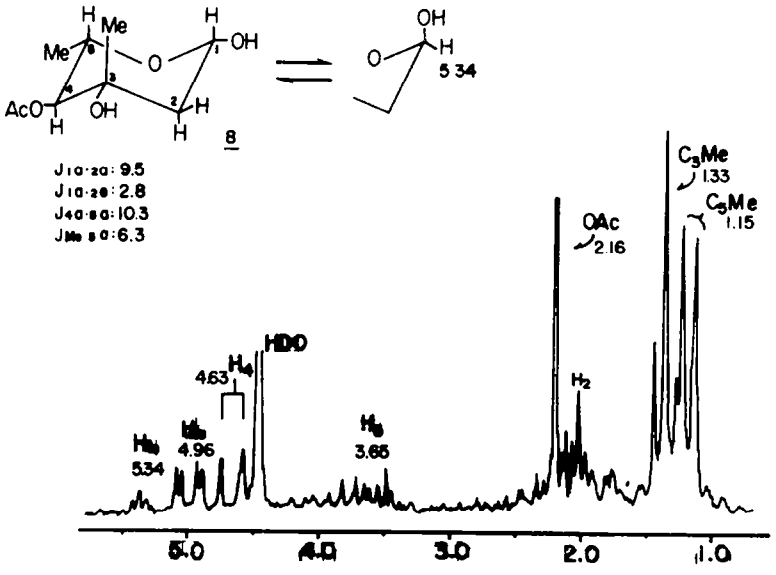


FIG. 7. NMR spectrum of chromose B (8) in D_2O measured at 80°, ppm from DSS, 60 Mc.

Chromose B has an acetoxyl group whose point of attachment can be readily determined by comparing the NMR spectrum of deacetylchromose B with that of chromose B (Fig. 7). In the spectrum measured at ca. 35° the absorption of the proton alpha to the acetoxyl group is hidden by the large HDO. However, when the HDO signal is shifted by measurement at elevated temperatures, the absorption of the acetoxy carbinyl proton clearly appears as a doublet at 4·63 with a coupling constant of 10·3 c/s. Since this doublet corresponds to the C_3-proton signal located at 3·26 ppm in the spectrum of deacetylchromose B (Fig. 6), it is clear that the acetoxyl group is attached to C_4. This conclusion is further supported by the fact that L-chromose B istelf does

[15] P. P. Regna, F. A. Hochstein, R. L. Wagner, Jr. and R. B. Woodward, *J. Amer. Chem. Soc.* **75**, 4625 (1953).

[16] R. Corbaz, L. Ettlinger, E. Gallmann, W. Keller-Schierlein, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, A. Wettstein and H. Zahner, *Helv. Chim. Acta* **39**, 304 (1956).

[17] R. Paul and S. Tschelitcheff, *Bull. Soc. Chim. Fr.* **5**, 443 (1957).

[18] R. B. Morin and M. Gorman, *Tetrahedron Letters* 2339 (1964).

[19] F. Korte, U. Claussen and K. Gohring, *Tetrahedron* **18**, 1257 (1962).

[20] D. M. Lemal, P. D. Pacht and R. B. Woodward, *Tetrahedron* **18**, 1275 (1962).

not consume periodic acid. The relative amount of the α and β anomers, as judged from the intensities of the H_{1e} and H_{1a} peaks is roughly 1:4.

D-*Chromose* C (2,6-*dideoxy*-D-arabino-*hexopyranose*)

Chromose C (**10**), $C_8H_{12}O_4$, was obtained as an oil by evaporation of the solvent from the last eluate of the column and could not be crystallized. Since the rotation

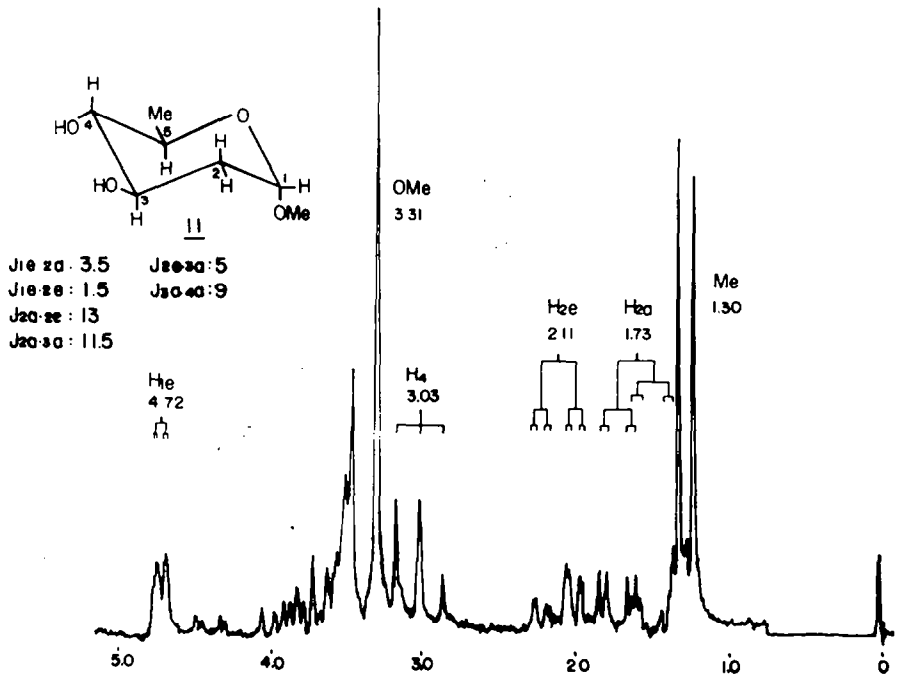
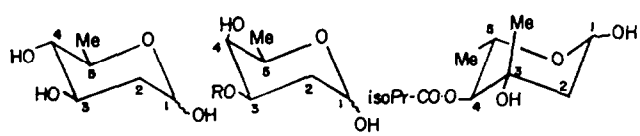


FIG. 8. NMR spectrum of methyl chromoside C (**11**) in $CDCl_3$, ppm from TMS, 60 Mc.



remained constant at $[\alpha]_D^{21} = +25°$ ($c = 1{\cdot}4$ in H_2O), the oil was already an equilibrium mixture of anomers. Periodic oxidation of chromose C yielded acetaldehyde and formic acid, while treatment with methanolic 5% hydrogen chloride afforded methyl chromoside C (**11**), $C_7H_{14}O_4$. The NMR spectrum of this glycoside **11** is shown in Fig. 8.

The quartet at 4·72 is assignable to an anomeric proton C_1–H, and the coupling constants obtained from this signal are 1·5 and 3·5 c/s, which correspond to equatorial–equatorial and equatorial–axial coupling constants, respectively. These values are substantiated by the absorption due to the C_2-methylene group. The splitting of the anomeric signal indicates that C_1–H is equatorial and that chromose C also has a

2-deoxypryanose structure. The absorption due to the C_2-methylene group gives a complex pattern around 2 ppm from which the chemical shift of H_{2a} and H_{2e} is shown to be 1·73 and 2·11, respectively, along with a geminal coupling constant of 13 c/s. H_{2a} and H_{2e} are further coupled to the proton at C_3 with coupling constants of 11·5 c/s (axial–axial) and 5·0 c/s (equatorial–axial). The peak due to H_4 is located at 3·03 as a triplet with spacings of 9 c/s (axial–axial), which is caused by its coupling to two adjacent protons at C_3 and C_5. This establishes that all three protons attached to C_3, C_4 and C_5 are axial and that the methyl at C_5 is equatorial.

The fact that chromose C, which is only obtained as an oil, is already an equilibrium mixture is clearly shown by its NMR spectrum taken in heavy water. Namely, there appear two quartets of roughly equal intensity at 4·90 ($J_{1a\ 2a}$: 10 c/s, $J_{1a\ 2e}$: 4 c/s) and 5·33 ($J_{1e\ 2e}$: 1·5 c/s, $J_{1e\ 2a}$: 4 c/s) which can be assigned to the two anomers with their C_1-protons respectively axial and equatorial.

This leads to a 2-deoxyrhamnose structure for the sugar. The rotation of the anomeric mixture from 2-deoxy-L-rhamnose is $[\alpha]_D^{14} = -18{\cdot}2°$,[21] which is opposite to that of the present sugar. Application of Whiffen's calculation[12] to methyl chromoside C gives a value of $[M]_D = +202°$ for the D-series as compared to the observed molecular rotation $[M]_D = +141°$. Both observations indicate that chromose C belongs to the D-series of sugars.

D-*Chromose* D (2,6-*dideoxy*-3-O-*acetyl*-D-lyxo-*hexopyranose*)

Chromose D (**12**) was obtained as crystals, $C_8H_{14}O_5$, $\nu_{cm^{-1}}^{CHCl_3}$:1735 (OAc), $\delta_{ppm}^{D_2O}$ 2·13 (OAc), 1·21 (sec-Me).

Upon treatment with potassium carbonate, it afforded acetic acid and deacetylchromose D (**13**), $C_6H_{12}O_4$. On the other hand, demethylation of D-chromose A with boron trichloride[22] afforded demethyl-D-chromose A, which was identical with deacetylchromose D in every respect. Chromose D has an acetoxyl group which, from the fact that chromose D itself consumes one mole of periodic acid, must be attached to C_3. The correlation between chromose D and D-chromose A establishes that chromose D also belongs to the D-series.

L-*Chromose* B′ *or* 4-O-*isobutyryldeacetylchromose* B (2,6-*dideoxy*-3-C-*methyl*-4-O-*isobutyryl*-L-arabino-*hexopyranose*)

The lipid-soluble fraction resulting from the hydroylsis of chromomycin A_2[1b] with methanolic hydrogen chloride at room temperature contained a methyl glycoside (tentatively named chromoside B′[1b]) in addition to the chromophore, chromomycinone.[2] The syrupy glycoside, which was homogenous on thin-layer chromotogram, was hydrolysed with sodium hydroxide to give isobutyric acid (identified as *p*-bromophenacyl ester) and methyl deacetylchromoside B. The NMR spectrum of the original glycoside showed a one-proton doublet (J 10·5 c/s) at 4·73 ppm due to the C_4–H; comparison of this chemical shift with those of the corresponding protons in deacetylchromose B (3·26 ppm, Fig. 6) and chromose B (4·63 ppm, Fig. 7) established that chromose B′ was 4-O-isobutyryldeacetylchromose B (**14**).

[21] B. Iselin and T. Reichstein, *Helv. Chim. Acta* **27**, 1146 (1944).

[22] S. Allen, T. G. Bonner, E. J. Bourne and N. M. Saville, *Chem. and Ind.* 630 (1958); T. G. Bonner, E. J. Bourne and S. McNally, *J. Chem. Soc.* 2929 (1960).

EXPERIMENTAL

The IR spectra were taken with a Hitachi EPI-S2 instrument. UV spectra were recorded with a Hitachi EPS-2 model and, unless otherwise stated, for EtOH solutions. NMR spectra were taken with Varian A-60 and HR-100 models, the chemical shifts being expressed in ppm relative to internal TMS for $CHCl_3$ solutions and internal DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) for aqueous solutions. M.ps are uncorrected.

The acid hydrolysis of chromomycin A_3

Chromomycin A_3 (15 g) dissolved in 50% AcOH (200 ml) was allowed to stand for 30 hr at 65°. The reaction mixture was evaporated to a dark residue, which solidified with foaming when the AcOH was completely removed under red. press. The resulting solid was dissolved in water and filtered from insoluble material; the filtrate was treated with active carbon and then evaporated to a syrupy residue (8 g), which showed the following spots on a paper partition chromatogram when developed with n-butanol saturated with water, and sprayed with *m*-aminophenol[23] (Fig. 9).

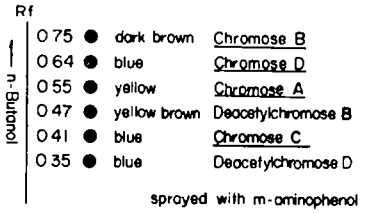


FIG. 9. The paper chromatogram of the chromoses.

The syrup was chromatographed on cellulose powder (Toyo roshi, mesh 100–200, column 7 cm × 150 cm) and eluted with cyclohexane and AcOEt (4:1 *v/v* gradually changed to 100% AcOEt), when the following fractions were obtained; fractions 1 and 7 were the first and last fractions, respectively, to be eluted from the column.

(1) chromose B (oil) 2 g.
(2) mixture of chromose B and chromose D (oil) 0·3 g.
(3) chromose D (crystals m.p. 118°) 1·0 g.
(4) mixture of chromose D and chromose A (oil) 0·2 g.
(5) chromose A (crystals, m.p. 153°) 1·6 g.
(6) mixture of chromose A and chromose C (oil) 0·4 g.
(7) chromose C (oil) 1·5 g.

Chromose A (**1**)

Recrystallization from ethanol gave needles, m.p. 153°, $[\alpha]_D^{23} = +93°$ ($c = 1{\cdot}0$ in H_2O) immediately after preparation, $[\alpha]_D^{23} = +77°$, after 1 hr or after 1 day. (Found: C, 52·01; H, 8·88; OMe, 19·57. $C_7H_{14}O_4$ requires: C, 51·85; H, 8·64; OMe, 19·13%.) ν_{max}^{KBr} 3300, 2900, 1450, 1230, 1080, 950, 880 cm^{-1}.

The needles gave positive Fehling and Webb–Levy[24] tests but did not consume periodic acid.

Methyl chromoside A (**4, 5**)

(a) *From chromose* A. Chromose A (1 g) was dissolved in 10 ml of a 5% methanolic HCl. The reaction mixture was allowed to stand overnight at room temp. and then passed through a column packed with IR-4B to remove the HCl. The eluent was evaporated to a solid, which was extracted with ether and separated into two fractions; one was easily soluble and the other only slightly soluble in ether. Each fraction was evaporated and recrystallized from cyclohexane. Methyl

[23] S. Hirase, C. Araki and S. Nakanishi, *Bull. Chem. Soc. Japan* **26**, 183 (1953).
[24] J. M. Webb and H. B. Levy, *J. Biol. Chem.* **213**, 107 (1955); W. Nagata, Ch. Tamm and T. Reichstein, *Helv. Chim. Acta* **40**, 41 (1957).

α-D-chromoside A (**4**), needles from the ether-soluble fraction, m.p. 92°, $[\alpha]_D^{22} = +122°$ ($c = 1·0$ in EtOH). (Found: C, 54·34; H, 8·80; OMe, 34·93. $C_8H_{16}O_4$ requires: C, 54·54; H, 9·09; OMe, 35·22%.) ν_{max}^{KBr} 3300, 2900, 1400, 1340, 1200, 1050, 920 cm^{-1}. Methyl β-D-chromoside A (**5**), needles from the relatively insoluble fraction, m.p. 152° $[\alpha]_D^{24} = -36°$ ($c = 1·0$ in EtOH). (Found: C, 54·24; H, 8·94; OMe, 35·49. $C_8H_{16}O_4$ requires: C, 54·54; H, 9·09; OMe, 35·22%.) ν_{max}^{KBr} 3300, 2900, 1400, 1340, 1200, 1050, 920 cm^{-1}.

Methyl α-D chromoside A (200 mg) was dissolved in a mixture of acetic acid (0·2 ml) and water (3 ml), and warmed on the water bath for 30 min. The reaction mixture was evaporated *in vacuo* to a solid. Recrystallization from AcOEt gave needles which were identical with D-chromose A. The β-anomer also gave D-chromose A upon similar treatment.

(b) *From chromomycin* A_3. A solution of chromomycin A_3 (15 g) in 5% methanolic HCl (150 ml) was allowed to stand for 2 hr at room temp. The reaction mixture was passed through a column packed with IR-45 to remove the HCl. The solution was evaporated, treated with water, and the insoluble material was removed by filtration. The aqueous solution was clarified with active C and evaporated to afford an oil (8 g), which was chromatographed on alumina. Elution with AcOEt followed by evaporation yielded crude crystals. These crude crystals were treated with ether, when the more soluble portion afforded methyl α-D-chromoside A (**4**) while the less soluble portion gave the β-anomer **5**; both chromosides were recrystallized from cyclohexane. The column of alumina was then eluted with MeOH, the combined eluate was evaporated and the residue was hydrolysed (to remove methoxyl groups from the sugars) by warming in 10% AcOH for 30 min at a bath temp of 70–80°. The solvent was evaporated, and after treatment of the residue with a small amount of water, the syrup was chromatographed on cellulose powder and developed with cyclohexane and AcOEt to yield chromose C, deacetylchromose B and deacetylchromose D.

2,4-*Dinitrophenylhydrazone of chromose* A (**2a**)

Chromose A (80 mg) dissolved in a small amount of EtOH was added to a solution of 2,4-dinitrophenylhydrazine (100 mg) in EtOH (4 ml) and conc. HCl (0·4 ml). The reaction mixture was set aside at room temp and the yellow-red crystals were filtered. Recrystallization from EtOH afforded crystals, 100 mg, m.p. 148–149°. (Found: C, 48·13; H, 4·93; N, 17·39; OMe, 9·81. $C_{13}H_{16}N_4O_6$ requires: C, 48·15; H, 4·97; N, 17·28; OMe, 9·57%.) ν_{max}^{KBr} 3400, 3250, 1618, 1595, 1520, 1338, 1142 cm^{-1}. λ_{max} (log ε) 374 mμ (4·49). $\lambda_{max}^{0.25N\ NaOH-EtOH}$ (log ε) 460 mμ (4·48).

Bromine oxidation of chromose A *to the lactone* **3**

Bromine (1·5 ml) was added with stirring to a solution of 4 g chromose A in 40 ml water. The reaction mixture was set aside overnight at room temp, and was then neutralized with Ag_2CO_3. The excess silver ion was precipitated by passing H_2S gas. After treatment with C, the solution was evaporated to an oil, the water being completely removed under red. press. Distillation of the oil gave the lactone, 2·8 g, b.p. 169–172°/2 mm. (Found: C, 52·68; H, 7·71; OMe 18·86; CMe, 12·12. $C_7H_{12}O_4$ requires: C, 52·49; H, 7·55; OMe, 19·37; CMe, 9·38%.) $\nu_{max}^{liq.}$ 3500, 1733, 1090 cm^{-1}.

This lactone gave a negative iodoform test; however, after hydrolysis with 20% NaOHaq the iodoform test became positive.

L-*Chromose* B (**8**)

The residual oil obtained by evaporation of the solvent under red. press. of the first eluate of the chromatogram (see above) was designated chromose B; $[\alpha]_D^{21} = -26°$ ($c = 1·1$ in H_2O) immediately after preparation of solution, $[\alpha]_D^{21} = -24°$, after 40 min and longer. Chromose B did not consume periodic acid. (Found: C, 53·02; H, 8·19; OAc, 22·54. $C_9H_{16}O_5$ requires: C, 52·92; H, 7·84; OAc, 21·09%.) $\nu_{max}^{CHCl_3}$ 3450, 1733, 1250, 1210, 1050 cm^{-1}.

Deacetylchromose B (**9**)

Chromose B (1 g) was dissolved in 30 ml of 2% K_2CO_3aq and allowed to stand overnight at room temp. The solution was passed through a column of IR-120 and then through a column of IR-4B, and was evaporated to a solid *in vacuo*. Recrystallization from EtOH gave crystals, 0·7 g, m.p. 109°; $[\alpha]_D^{22} = -15°$ ($c = 1·0$ in H_2O) immediately after preparation, $[\alpha]_D^{22} = -22°$, after 1 hr and longer. (Found: C, 51·59; H, 8·69; CMe, 18·19. $C_7H_{14}O_4$ requires: C, 51·84; H, 8·70; CMe, 18·51%.) ν_{max}^{KBr} 3400, 1420, 1350, 1080, 985, 850 cm^{-1}. Deacetylchromose B gave positive Fehling and iodoform tests.

The acid fraction absorbed on IR-4B was eluted with 2% NH_4OH. The eluate was evaporated to dryness under red. press. when the residue was identified as $AcONH_4$ by paper chromatography.[25]

Methyl deacetylchromoside B

Deacetylchromose B (2 g) dissolved in 30 ml 5% methanolic HCl was left in the ice-box overnight, and the acid was removed by passage through a column of IR-4B. The eluate was concentrated and distilled to give 1 g of methyl deacetylchromoside B, b.p. 140°/10 mm. (Found: C, 53·95; H, 9·34; OMe, 17·50. $C_9H_{16}O_4$ requires: C, 54·54; H, 9·09; OMe 17·61%.)

D-*Chromose* C (10)

The oil obtained by evaporation (red. press.) of the last eluate from the chromatogram (see above) was designated chromose C; $[\alpha]_D^{21} = +25°$ ($c = 1·4$ in H_2O). Chromose C gave positive Fehling and Webb–Levy[24] tests. (Found: C, 48·73; H, 8·18; OMe, 11·03. $C_6H_{12}O_4$ requires: C, 48·64; H, 8·11; OMe, 10·13%.) ν_{max}^{Nujol} 3400, 1455, 1380, 1065, 990 cm^{-1}.

Methyl chromoside C (11)

Chromose C (1 g) was dissolved in 5% methanolic HCl (10 ml) and the reaction mixture was allowed to stand at room temp. The solution was passed through a column of IR-45 and was evaporated to an oil *in vacuo*. Distillation of the oil gave methyl chromoside C, 0·8 g, b.p. 126–129°/1·0 mm; $[\alpha]_D^{23} = +87°$ ($c = 1·2$ in H_2O). (Found: C, 51·85; H, 8·96; OMe, 19·18. $C_7H_{14}O_4$ requires: C, 51·85; H, 8·64; OMe, 19·13%.) $\nu_{max}^{CHCl_3}$ 3400, 1450, 1390, 1210, 1130, 1060, 980 cm^{-1}.

D-*Chromose* D (12)

Evaporation of the solvent from the third fraction of the chromatogram (see above) afforded needles, m.p. 118°; $[\alpha]_D^{23} = +87°$ ($c = 1·5$ in H_2O) 5 min after preparation of solution. Chromose D gave positive Fehling, Kiliani,[26] Webb–Levy,[24] and diphenylamine reactions. (Found: C, 50·68; H, 7·31; OAc, 23·51. $C_8H_{14}O_5$ requires: C, 50·52; H, 7·36; OAc, 22·63%.) $\nu_{max}^{CHCl_3}$ 3450, 1735, 1370, 1240, 1080, 1040, 980 cm^{-1}.

Deacetylchromose D (13)

Chromose D (150 mg) was dissolved in 3% K_2CO_3aq and was allowed to stand for 2 days at room temp. The solution was passed through a column packed with IR-120, and then through a column packed with IR-45. The eluate was concentrated to a syrup (80 mg); $[\alpha]_D^{20} = +53°$ ($c = 1·2$ in H_2O). (Found: C, 45·62; H, 8·28. $C_6H_{12}O_4 \cdot \frac{1}{2}H_2O$ requires: C, 45·89; H, 8·28%.) $\nu_{max}^{liq.}$ 3400, 1460, 1380, 1250, 1070, 990 cm^{-1}.

The acid portion absorbed on IR-45 was eluted with 2% NH_4OH, and the eluate was concentrated to a small volume. AcOH was detected in the concentrate by paper chromatography.

Demethylation of chromose A

BCl_3 (25 g) was added slowly at −70° to a solution of chromose A (400 mg) in CH_2Cl_2 (40 ml), and the mixture was kept at −70° for 30 min. After removal of excess BCl_3 at room temp and evaporation of solvent, the residual oil was treated with MeOH (10 ml), and once again taken to dryness. The residual oil, dissolved in MeOH, was passed through an IR-45 column. The eluate was condensed to an oil which was heated with 10% AcOH. The solution was evaporated to a syrup, which was identified as being deacetylchromose D by means of IR, NMR and paper partition chromatography.

Oxidation of chromoses with periodate

(a) *Preparation of sample solution.* Each sugar (0·1 mM) was dissolved in 20 ml of 0·1 M acetate buffer (pH 4·6) and 20 ml of 0·02 M potassium periodate aq. The solution was allowed to stand at 0–5°. To determine the consumption of periodate, aliquots of 3 ml were taken out from the reaction mixture and titrated with a standard iodine solution.

[25] E. P. Kennedy and H. A. Barker, *Anal. Chem.* **23**, 1033 (1951).
[26] H. Kiliani, *Arch. Pharm.* **251**, 567 (1913).

(b) *Blank test.* Saturated $NaHCO_3$aq (10 ml), 0·01 M sodium arsenite aq (5 ml) and 20% KIaq (1 ml) were quickly added to a mixture of 0·02 M potassium periodate (1·5 ml) and 0·1 M acetate buffer (1·5 ml). The solution was set aside for 15 min at the temp described above. After adding 1% starch solution (1 ml), the solution was titrated with 0·01 M iodine until the violet color persisted for 10 sec.

(c) *Tests.* Aliquots of 3 ml were taken from the reaction mixture at suitable intervals. Each aliquot was treated with saturated $NaHCO_3$aq (10 ml), 0·01 M sodium arsenite (5 ml) and 20% KI (1 ml), and after 15 min, the solution was titrated with 0·01 M iodine solution in the presence of starch. The periodate consumption was estimated by subtraction of the blank from the value obtained in the above titration.

(d) *Detection of formic acid.* The reaction mixture was acidified with HCl and extracted with ether. This ether layer was extracted with 5% NH_4OH, the ammonia solution was condensed to a small volume, and the concentrated solution was applied to a paper chromatogram using 95% EtOH/ammonia (100/1) as developing agent and $AgNO_3$ as coloring agent.

(e) *Detection of acetaldehyde.* Alcoholic 2, 4-dinitrophenylhydrazine in 2N HCl was added to the ether extract which had been extracted with ammonia for detection of formic acid. The ether solution was evaporated to a gummy substance, from which acetaldehyde was separated as its 2,4-dinitrophenylhydrazone, and identified by IR spectrum, m.p. and paper chromatography; the paper chromatrogram was pre-treated with 20% alcoholic dimethyl formamide and developed with cyclohexane.

(f) *Determination of acetaldehyde.* The aqueous solution of the sugar was treated with periodic acid at 50–60°. N_2 was bubbled through the solution during the reaction in order to introduce the acetaldehyde into an EtOH–HCl solution of 2,4-dinitrophenylhydrazine. The hydrazone was collected and weighed.

	Periodate consumption (M)					
	15 min	1 hr	3 hr	24 hr	formic acid	acetaldehyde
Chromose B (**8**)	0	0	0	0·06	−	−
Deacetylchromose B (**9**)	0·5	2·13	2·2	2·6	+	0·95M
Chromose C (**10**)	2·1	2·5	2·5	3·8	+	0·89M
Methyl chromoside C (**11**)	0·9	1·1	1·1	1·3	−	−
Chromose D (**12**)	0·06	0·26	0·8	1·5	−	+

Methanolysis of chromomycin A_3

Chromomycin A_3 (2 g) was dissolved in 50 ml of 5% methanolic HCl and left at room temp for 3 hr. The hydrolysis was stopped when TLC had shown that no starting material was left. The reaction mixture was poured into saturated NaClaq and extracted thoroughly with AcOEt. The extract was washed with water, concentrated, and the residue was treated with $CHCl_3$. The precipitate was filtered and recrystallized from AcOH to afford chromomycinone. The mother liquor was chromatographed on a column of active charcoal and eluted with benzene when 250 mg of an oil was obtained. The oil could not be induced to crystallize but gave only a single spot on TLC. The oil was identified as methyl 4-O-isobutyryldeacetyl-α-chromoside B (**15**) from analyses, spectroscopic data, and hydrolysis results (see following). (Found: C, 58·58; H, 8·92; OMe 12·29. $C_{18}H_{32}O_8$ requires: C, 58·53; H, 8·94; OMe, 12·60%.) ν_{max}^{chf} 3500, 2950, 1730, 1450, 1395, 1190, 1155, 1130, 1050 cm^{-1}. NMR (in $CDCl_3$, ppm from TMS) δ 1·20 (3H, doublet, J 7·0, C_5–Me), 1·21 (6H, doublet, J 7·0, isopropyl methyls), 1·38 (3H, singlet), 2·67 (1H, septet, J 7·0, isopropyl methine), 3·36 (3H, singlet, OMe), 4·73 (1H, doublet, J 10·5, H_{4a}), 4·80 ppm (1H, triplet, J 3·0, H_{1e}); the NMR spectrum showed that glycoside was contaminated with a small amount of the β-anomer.

Hydrolysis of methyl 4-O-*isobutyryldeacetylchromoside* B (**15**)

The glycoside (450 mg) dissolved in 5 ml EtOH was treated with 5 ml 1N NaOH and the solution was left overnight at room temp. The solvent was removed and the residue was extracted thoroughly with ether. Evaporation of the ether afforded methyl deacetylchromoside B (identified by means of

IR). The sodium salt that was left from the ether extraction was dissolved in the minimum amount of water, neutralized with dil. HCl, treated with 350 mg of *p*-bromophenacyl bromide, and boiled for 2 hr on the water bath while adding EtOH so that the solution remained clear. The reaction mixture was then extracted twice with $CHCl_3$, and the extract was concentrated after water-washing and drying. The residue was dissolved in a 3:1 mixture of CCl_4 and benzene and passed through a 75 × 1·2 cm column of Merck silica gel, when the phenacyl ester was eluted after a considerable amount of fore-run. The phenacyl ester was recrystallized from EtOH to give needles, m.p. 77°, which were identified as isobutyryl *p*-bromophenacyl ester by comparison of m.p., IR and NMR with an authentic sample.

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