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THE FOUR CHROMOSES FROM CHROMOMYCIN A_3

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Chromomycin is the name given to a group of cancerostatic antibiotics produced by Streptomyces griseus No.7, and is a mixture of several closely related compounds[1]. The principal constituent, which is commercially available, has been designated chromomycin A_3[1]. The isolation of closely related compounds has been reported by several groups: e.g., aureolic acid[2], aburamycin[3], LA-7017[4], M5-18903[5], NSCA-649[6], mithramycin[7], olivomycin[8].

These antibiotics apparently belong to a new class of compounds, but their structures remain unclarified. This and following communications[9),10] deal with the structural elucidation of chromomycin A_3, $C_{51}H_{72}O_{23}$.

Chromomycin A_3 can be hydrolysed to the four 2,6-dideoxy-sugars, chromoses A[11], B, C and D, and the aglycone chromomycinone[9] by boiling in 50% aqueous acetic acid. Chromose A has been established[11] to belong to the D-series and its

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structure along with those of chromoses B, C and D is shown in FIG.1.

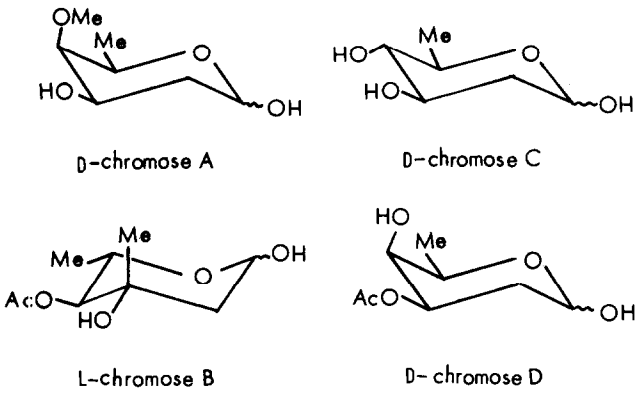

FIG.1.

2,6-Dideoxy-3-C-methyl-4-O-acetyl-L-arabo-hexopyranose (L-chromose B), $C_9H_{16}O_5$, is an oil* with $\nu_{cm^{-1}}^{CHCl_3}$ 1733(OAc), and $[\alpha]_D^{21}$ = -26°(c = 1,1 in H_2O, immediately after preparation), and -24° after 40 min. or longer. Hydrolysis of chromose B with potassium carbonate gave acetic acid and deacetylchromose B, $C_7H_{14}O_4$, m.p. 109°, $[\alpha]_D^{22}$ = -15°(c = 1.0 in H_2O, immediately after preparation), $[\alpha]_D^{22}$ = -22°(after 1 hour and longer).

FIG.2 shows the 100 mc spectrum, including spin-decoupling data of deacetylchromose B dissolved in D_2O containing a small amount of sodium 2,2-dimethyl-2-silapentane-5-sulfonate the

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* The analyses of all compounds described in this communication agreed with the molecular formulae although some could not be obtained crystalline.

latter acting as internal reference*.

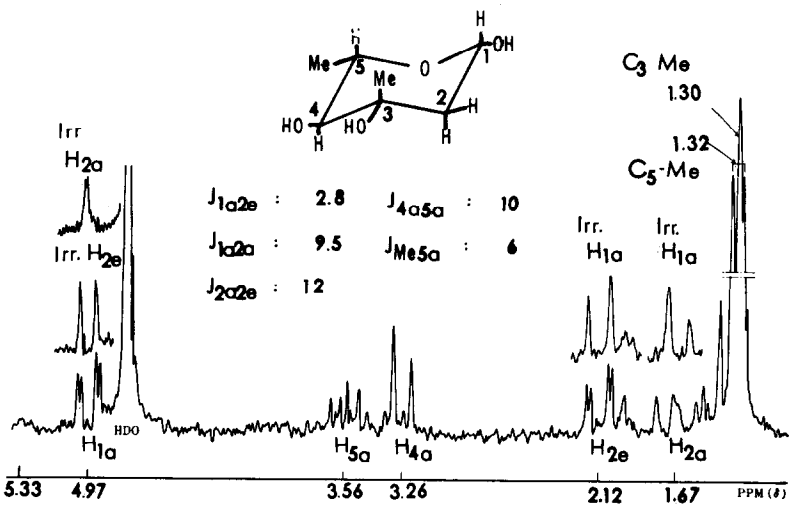

FIG.2 NMR spectrum and decoupled traces of deacetylchromose B in D_2O, ppm from DSS.

The NMR spectrum was measured immediately after dissolving the sample in D_2O. The tall peaks at 1.30 and 1.32 are due to C_3-Me and C_5-Me respectively. The latter is coupled with a spin coupling constant of 6 cps to its adjacent proton whose resonance appears at 3.56. H_5 is further coupled to H_4 with a characteristic axial-axial coupling constant of 10 cps.

The nonequivalent protons at C_2 resonate at 1.67 and 2.12, and exhibit a geminal coupling constant of 12 cps. C_2-H_a and C_2-H_e are further coupled to C_1-H with coupling constants of 9.5(axial-axial) and 2.8(axial-equatorial) cps. The peaks

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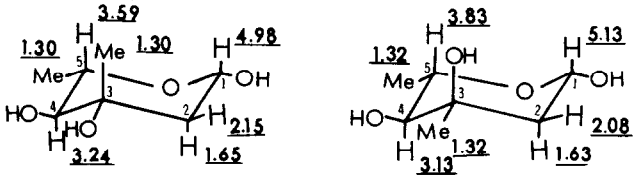
* See Preface II in N.S.Bhacca, D.P.Hollis, L.F.Johnson and E.A.Pier, "NMR Spectra Catalog" Volume 2. Varian Associates 1963.

corresponding to C_1 is located at 4.97 and the width of resonance pattern is 13 cps. However, after three days, the intensity of the quartet at 4.97 is reduced and a triplet (5 cps wide) of corresponding intensity is observed at 5.33. This indicates that axial-axial and axial-equatorial couplings of 9.5 and 2.8 has now been supplemented by a pattern which has axial-equatorial and equatorial-equatorial couplings of 2.5 cps. Thus anomerization takes place at C_1.

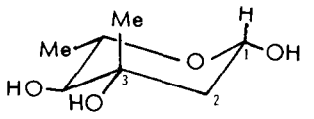


3-epimycarose mycarose

FIG.3 The chemical shifts[12] (in ppm) calculated using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal reference.

The NMR spectrum of deacetylchromose B is very similar to that of mycarose[12] and 3-epimycarose[12] which are quite similar to each other (see FIG.3). Comparisons of FIG.2 and 3 indicate that deacetylchromose B has the 3-epimycarose structure, namely 1, or 2. The negative direction of mutarotation in deacetylchromose B (-15° to -22°) is accompanied by the change in NMR signals of the anomeric C_1-proton (axial to equatorial). Thus deacetylchromose B belongs to the L-series and is the yet unknown[13] 3-epi-L-mycarose.

Chromose B has an acetate group. The attachment of this function can be readily determined by comparing the NMR spectra



1 β-L
(more positive anomer)

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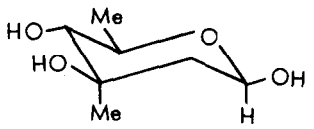


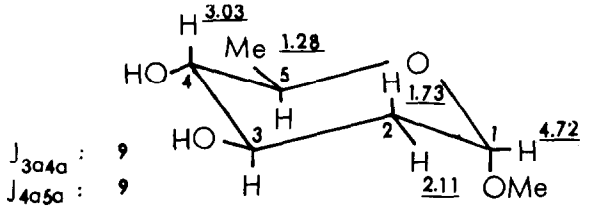
2 β-D
(more negative anomer)

of deacetylchromose B with that of chromose B. The signals corresponding to the proton at C_4 shift down from 3.26 to 4.63 due to the replacement of hydroxyl by a more electronegative acetoxyl group. Therefore the acetate group must be located at C_4 in the equatorial position. This is further substantiated by the fact that L-chromose B itself does not consume periodic acid.

2,6-Dideoxy-D-arabo-hexopyranose(D-chromose C), the sugar eluted from the cellulose powder column was an oil that analysed for $C_6H_{12}O_4$; the $[\alpha]_D^{21}$ value of +22°(c = 1.4, in H_2O) remained constant, and thus the oil is already an equilibrium mixture of anomers. Its 60 mc NMR spectrum in D_2O solution showed two quartets of equal intensity at 5.33 ppm(J: 1.5, 4 cps) and 4.90 ppm(J: 2, 10 cps), which could be assigned to the equatorial and axial C_1-H, respectively. Periodic oxidation of the oil yielded acetaldehyde and formic acid. Treatment of the oil with 10% HCl-MeOH gave methyl chromoside C, $C_7H_{14}O_4$,

b.p.$_{1.0}$ 126-129°, $[\alpha]_D^{23}$ = +87°(c = 1.2 in H_2O).

The 60 mc spectrum of methyl chromoside C, dissolved in $CDCl_3$ containing a trace amount of tetramethylsilane, showed the following signals:



The resonance at 4.72 was assigned to the proton at C_1. It shows two small coupling constants (3.5 and 1.5 cps) to its adjacent methylene group protons. This establishes equatorial configuration for H_1. Since signals at C_4 exhibit two 9 cps couplings, all three protons at C_3, C_4 and C_5 must be axial. This leads to a 2-deoxyrhamnose structure for the sugar. The rotation of the anomeric mixture of 2-deoxy-L-rhamnose[14] is $[\alpha]_D^{14}$ = -18.2°, which is opposite that of the present sugar. Accordingly chromose C belongs to the D-series (see FIG.1).

2,6-Dideoxy-3-O-acetyl-D-lyxo-hexopyranose(D-chromose D), $C_8H_{14}O_5$, was obtained in the form of crystals, m.p. 128°, $[\alpha]_D^{23}$ = +87°(c = 1.5 in H_2O), $\nu_{cm^{-1}}^{CHCl_3}$ 1715(OAc), $\delta_{ppm}^{D_2O}$ 2.13(OAc), 1.21(sec-Me).

Chromose D consumes one mole of periodic acid. Upon treatment with potassium carbonate it gives acetic acid and deacetylchromose D, $C_6H_{12}O_4$, $[\alpha]_D^{20}$= +53°(c = 1.2 in H_2O); the latter is identical with demethyl D-chromose A(demethylation

with boron trichloride[15]). The structure of this sugar is thus also established (see FIG.1).

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