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STRUCTURES OF METHYL ALDGAROSIDES A AND B OBTAINED FROM THE NEUTRAL MACROLIDE ANTIBIOTIC ALDGAMYCIN E

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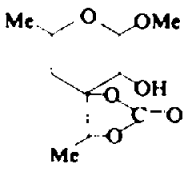
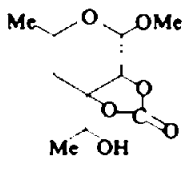
Abstract—The methanolysis of aldgamycin E yields, in addition to methyl mycinoside, two anomeric carbonate containing methyl glycosides, A and B. The structures of the two carbonate sugars have been elucidated and found to belong to the D series.

IN A preliminary report we recently described the nature of two carbonate methyl glycosides, methyl aldgarosides A(I) and B(II), obtained by methanolysis of aldgamycin E.[1] While continuing studies directed at the relative stereochemistry of these unusual sugars, it become necessary to reconsider the original interpretation of the NMR spectra and hence to reassign the structure of B to that represented by IV. It is the purpose of this paper to describe these supplementary considerations and, in addition, to present evidence for the stereochemistry of A and B as indicated in formulae III and IV.



I



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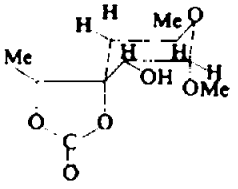
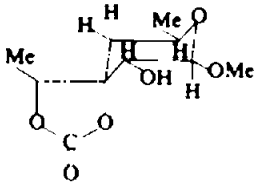


III



IV

Aldgamycin E, a neutral macrolide antibiotic isolated from the culture filtrate of *Streptomyces lavendulae*,[2] has an unusual absorption at 1800 cm^{-1} in its IR spectrum.[3] The antibiotic is stable to acid (0·1N HCl, 80°, 2 hr) but readily loses CO_2 when treated with base (0·1N NaOH, 80°, 2 hr) followed by acidification. To further investigate this phenomenon, aldgamycin E was hydrolyzed with a dilute solution

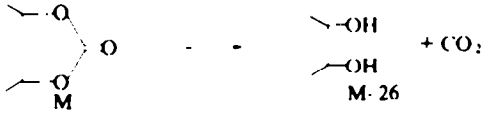
[1] M. P. Kunstmann, L. A. Mitscher and N. Bohonos, *Tetrahedron Letters* No. 8, 839 (1966).

[2] M. P. Kunstmann, L. A. Mitscher and E. L. Patterson, *Antimicrobial Agents and Chemotherapy* p. 87. Braun-Bromfield, Ann Abor, Michigan (1964).

[3] Two other neutral macrolide antibiotics, megacidin [L. Ettlinger, E. Gaümann, R. Hutter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog, P. Reusser and H. Zähner, *Monatsh. Chem.* **88**, 989 (1957)] and bandamycin [S. Kondo, J. M. J. Sakamoto and H. Yumoto, *J. Antibiotics, Ser. A*, **14**, 365 (1961)] also contain infrared absorption at 1800 cm^{-1}. Indeed, spectral properties suggest a close relationship of aldgamycin E to megacidin and bandamycin.

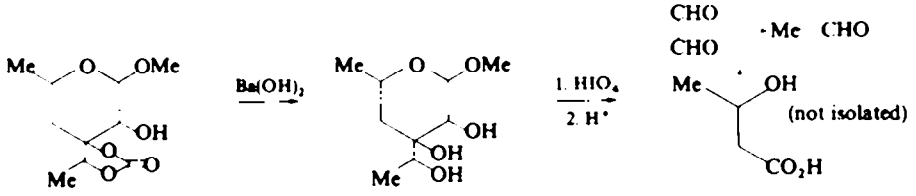
of barium hydroxide at room temperature. A precipitate of barium carbonate appeared almost immediately and the reaction product no longer exhibited IR absorption at 1800 cm^{-1}. Following purification, the product crystallized and was found to be identical (IR, UV, paper chromatography, m.p., and mixture m.p.) with a minor component isolated from culture AL471, aldgamycin C (AL471C, so named because it corresponded to the spot designated C in earlier paper chromatographic work).

These observations can all be rationalized satisfactorily by suggesting that E contains a 5-membered cyclic carbonate function. Cyclic carbonate groups are well known to be alkali labile but relatively stable to acid.[4] Additional support for the presence of the carbonate function in E and absence of it in C, is shown by the mol wts of E and C (742 and 716) as determined by direct inlet mass spectrometry. The difference between these two values (26) is consistent with the hydrolysis as shown below:



Methanolysis of aldgamycin E afforded three methyl glycosides. One was identified as methyl mycinoside (found also in chalcomycin[5] and neutramycin[6]) by comparison with an authentic sample. The other two methyl glycosides isolated contained the cyclic carbonate absorption in the IR spectrum and are evidently isomeric with each other. The IR spectrum of the less predominate isomer, methyl aldgaroside A, has carbonate absorption at 1800 cm^{-1}, whereas this absorption is observed at 1770 cm^{-1} in the spectrum of methyl aldgaroside B. Because of the predominance of methyl aldgaroside B from the methanolysis reaction, it was used in the structural studies and the two isomers related through their NMR spectra.

Hydrolysis of methyl aldgaroside B, $C_{10}H_{16}O_6$, *m/e* 232, with dilute barium hydroxide followed by periodate oxidation gave one mole each of acetaldehyde and glyoxal, isolated as their 2,4-dinitrophenylhydrazones.



The NMR spectrum of B (Fig. 1) in deuterochloroform, containing some deutero-dimethylsulfoxide, is in complete accord with formula IV and suggests the presence of one O-Me group (3-H singlet at 3·50 δ), and two secondary C-Me groups on carbons bearing an oxygen atom (two 3-H doublets at 1·52 and 1·23 δ, J = 6·0 c/s). The anomeric hydrogen signal occurs as a sharp doublet at 4·48 δ (J = 7·5 c/s) and

[4] L. Hough, J. E. Priddle and R. S. Theobald, *Advances in Carbohydrate Chemistry* Vol. 15; p. 93. Academic Press, New York (1960); L. Hough, J. E. Priddle, R. S. Theobald, G. R. Barker, T. Douglas and J. W. Spoors, *Chem. & Ind.* **148** (1960).

[5] H. W. Dion, P. W. K. Woo and Q. R. Bartz, *J. Am. Chem. Soc.* **84**, 880 (1962).

[6] M. P. Kunstmann and L. A. Mitscher, *Experientia* **21**, 372 (1965).

is fortuitously superimposed upon the C_7-H quartet. This apparent AB system was originally assigned to the H_1 and H_2 signals but analysis of the ratio of intensities to the separation of the peaks[7] negates this original interpretation as does deuterium exchange with CD_3OD. Hence, the one proton quartet at 4·48 δ (J = 6·5 c/s) produced by coupling with a Me group remains unchanged upon exchange with deuterium and is assigned to the C_7 proton. The C_2-H quartet at 3·30 δ (the fourth line is covered by the O-Me signal) is collapsed to a doublet upon exchange with CD_3OD consistent with the C_2-H coupled to the C_2-OH and the C_1 anomeric proton. The C_2-OH proton signal (1-H doublet at 5·30 δ) is lost upon deuterium exchange. Lack of additional coupling of the C_7 and C_2 proton signals confirms the absence of a hydrogen at C_3.

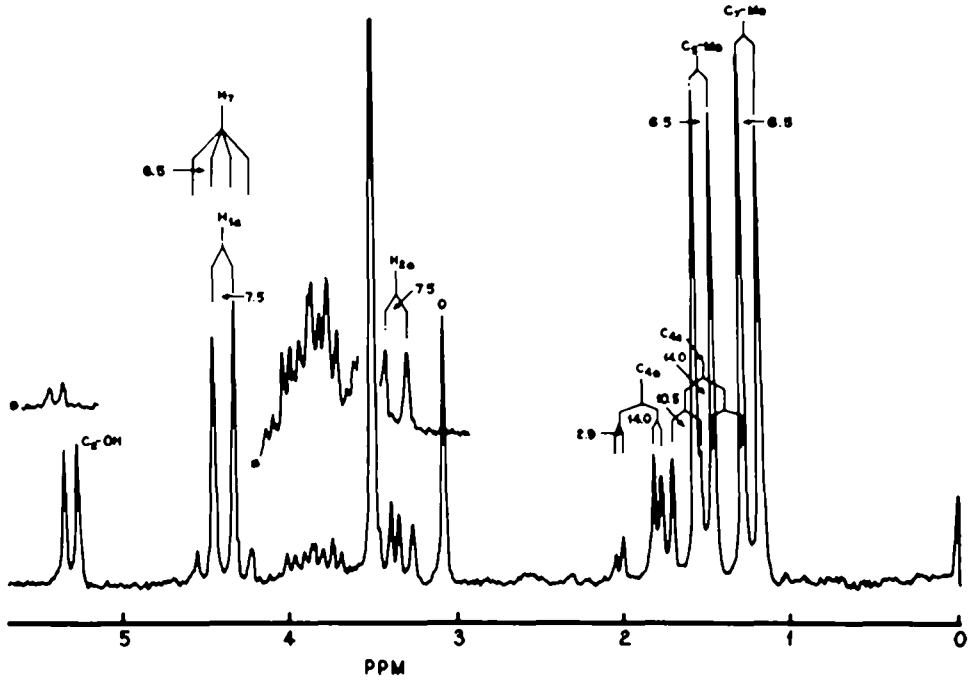


FIG. 1. NMR spectrum of methyl aldgaroside B. O resonance believed to be caused by an impurity: * after hydrogen exchange with CD_3OD.

The NMR spectrum of methyl aldgaroside A (Fig. 2) fully supports structure III and the anomeric nature of the two glycosides. Thus the C_1-H signal in A is shifted downfield to 4·70 δ (J = 4·5 c/s) in contrast to the corresponding signal in B (4·50 δ, J = 7·5 c/s) suggesting that in A the C_1 and C_2 hydrogens are in an equatorial-axial relationship whereas in B they are axial-axial.[8,9] The C_2-H is also shifted downfield in A to 3·67 δ and is spin-coupled to the C_2-OH (4·52 δ) as well as to the

[7] R. H. Bible, Jr., *Interpretation of NMR Spectra* p. 82. Plenum Press, New York, N.Y. (1965).

[8] J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.* **80**, 5121 (1958).

[9] J. N. Shoolery [*NMR and EPR Spectroscopy* p. 114, Varian Staff, Pergamon Press, New York, N.Y. (1960)] tabulates coupling constants for cyclohexane systems: $J_{a,a}$ = 6·14 c/s, $J_{a,e}$ or $J_{e,e}$ = 0–6 c/s. See also P. W. K. Woo, H. W. Dion and L. F. Johnson, *J. Am. Chem. Soc.* **84**, 1066 (1962) and R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *ibid.* **80**, 6098 (1958).

C_1-H. Upon deuterium exchange, the C_2-OH doublet disappears and the C_2-H quartet is collapsed to a doublet (J = 4·5 c/s).

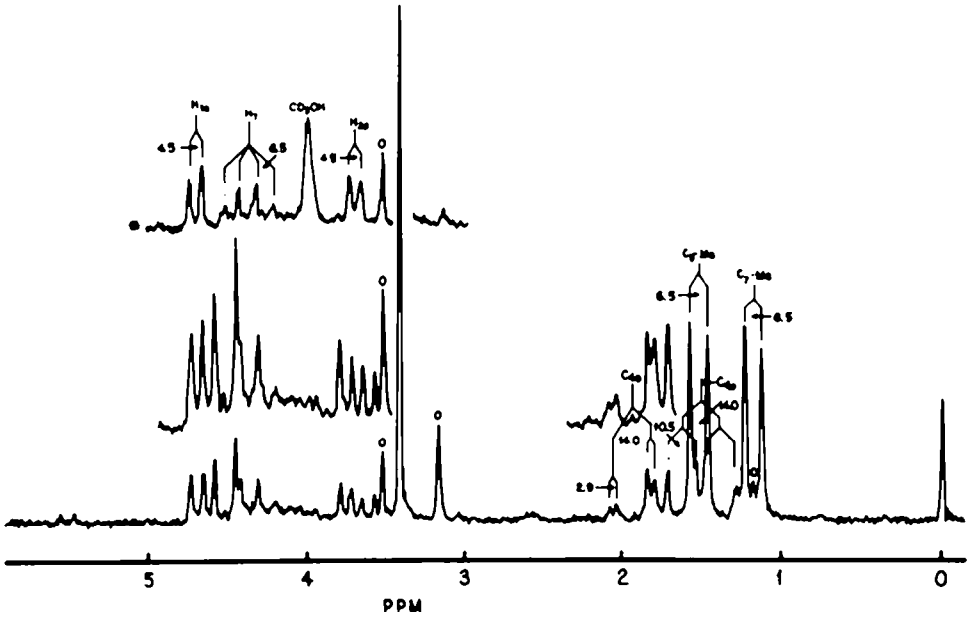


FIG. 2. NMR spectrum of methyl aldgaroside A. O resonance believed to be caused by an impurity; * after hydrogen exchange with CD_3OD

Although the above coupling constants for the C_1 and C_2 protons in A and B are indicative of the stereochemistry as shown, it remains possible that the cyclic carbonate group alters the normal pyranose bond angles by distortion of the ring from its usual conformation. However, examination of the NMR spectra of decarbonated A and B readily confirm the above conclusions (Figs 3 and 4). Thus, the C_{1e}-H signal in decarbonated A is found at 4·80 δ and has a coupling constant of 3·5 c/s whereas the C_{1a}-H signal in decarbonated B occurs at 4·50 δ with spin-coupling of 7·8 c/s.

The splitting patterns of the C_4 (1·80 δ) and C_5-H's (3·80 δ) are not completely clear in any of the above-mentioned spectra although a portion of the C_4-H signals can be assigned with reasonable certainty in the spectra of the two carbonate sugars. In order to obtain a more definitive picture of these signals, the NMR spectrum of the diacetate of decarbonated B (Fig 5) was examined. Irradiation of the low field doublet at 1·28 δ (J = 6·5 c/s), assigned to the C_5-Me group, collapsed the C_5-H signal to a distorted four line pattern because the upfield portion of the C_{4a}-H signal was also irradiated due to overlapping with the C_5-Me doublet. To determine the true coupling constants and pattern for the MNX portion of this A_3MNX system, the observed J values for the C_4-H signals ($J_{4a,4e}$ = − 14·0, $J_{4a,5a}$ = 10·8, and $J_{4e,5a}$ = 4·1 c/s) were incorporated into the Swalen–Reilly computer program.[10]

[10] J. D. Swalen and C. A. Reilly, *J. Chem. Phys* 37, 21 (1962).

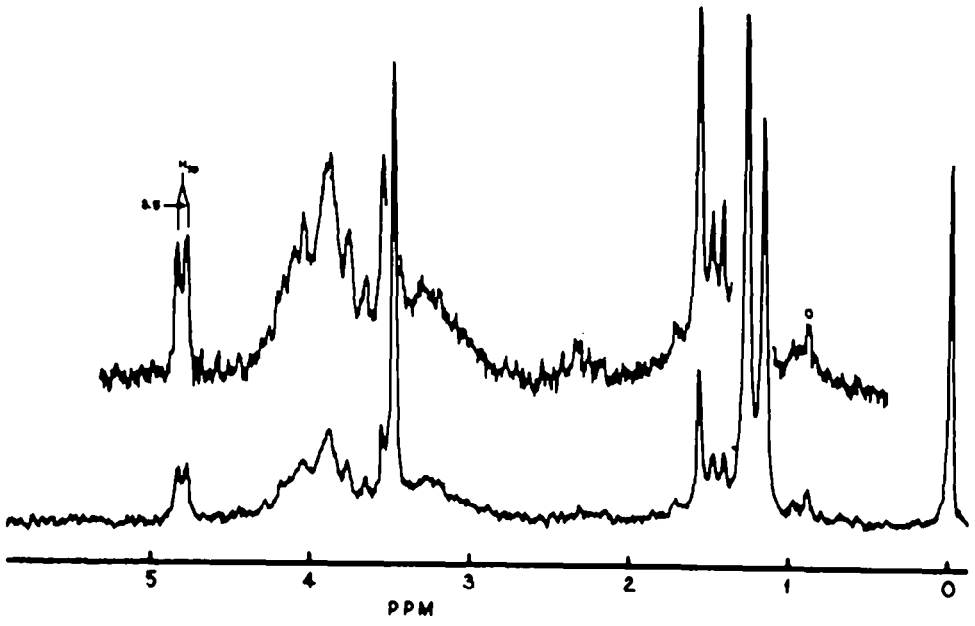


FIG. 3. NMR spectrum of decarbonated methyl aldgaroside A. * after hydrogen exchange with CD_3OD: O resonance believed to be caused by an impurity.

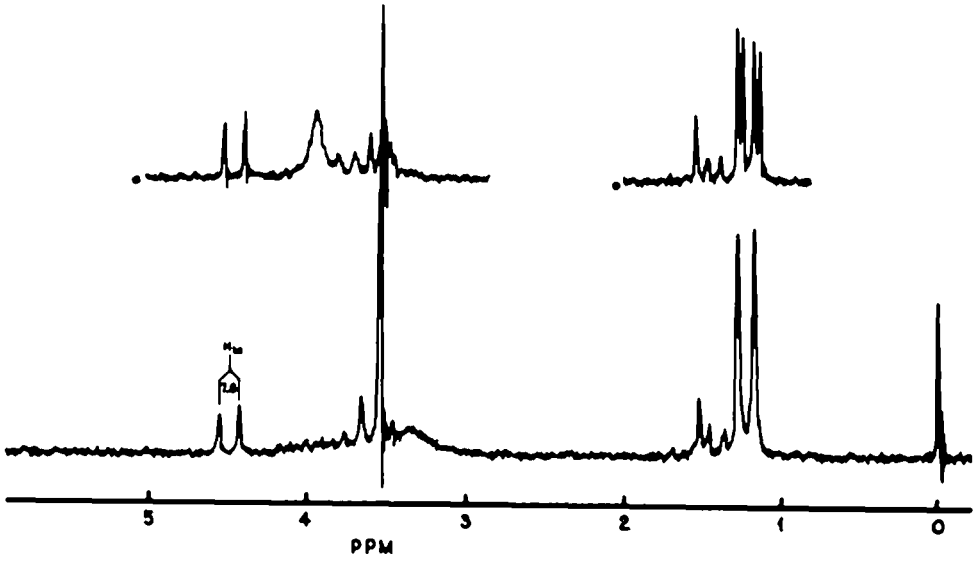


FIG. 4. NMR spectrum of decarbonated methyl aldgaroside B. * after hydrogen exchange with CD_3OD.

To simplify the calculations, the C_5-Me coupling effect was excluded from the program. The calculated values $J_{4a,4e} = -14{\cdot}0$, $J_{4a,5a} = 10{\cdot}8$, and $J_{4e,5a} = 2{\cdot}3$ c/s) clearly show the C_5 proton to be axial.[8,9] Using the computed spectrum, combined with the assumption that each one of the X lines should be a quartet with a J of 6·5 c/s (due to coupling with the C_5-Me group), the complete spectrum of the C_4 and C_5 protons is shown in the lower half of Fig. 5. From this experiment the C_4-H signals can be assigned unambiguously in the spectra of the carbonate sugars (Figs 1 and 2). It is also seen that the coupling constants for the C_4 protons agree with the calculated values for the diacetate of decarbonated methyl aldgaroside B.

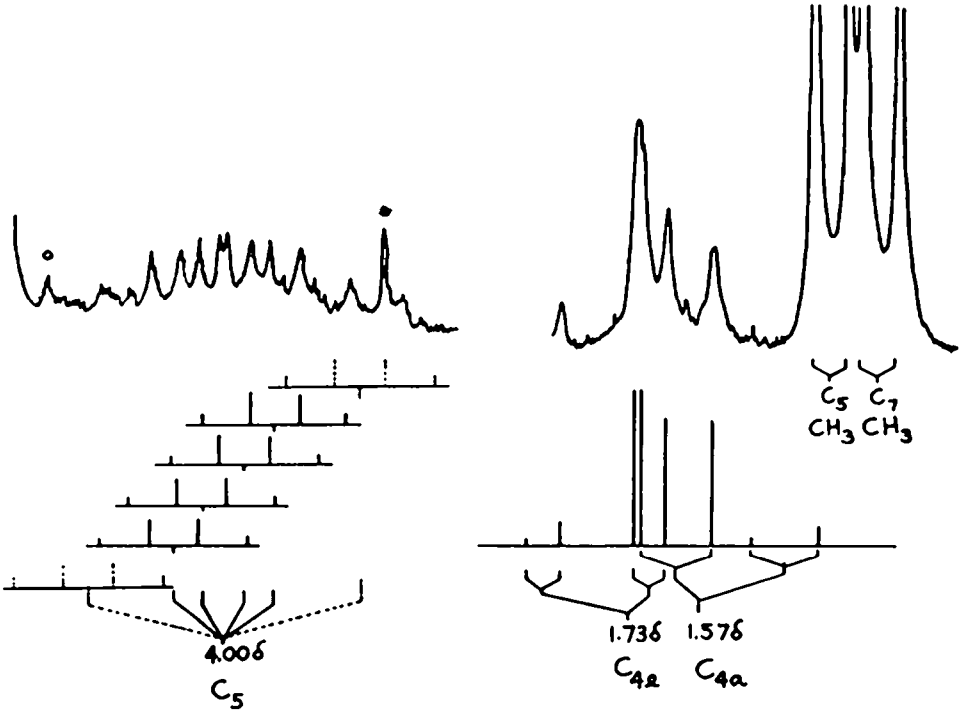


FIG. 5 Analysis of the C_4 and C_5 proton region of the NMR spectrum of the diacetate of decarbonated methyl aldgaroside B. O resonance believed to be caused by an impurity; * spinning side band. Dotted lines indicate unobserved signals. The C_5 proton signal is amplified 2½ times

At present there is no direct evidence for the stereochemistry at C_3 although examination of molecular models clearly show that the equatorial orientation of the ethyl side chain is the preferred conformation. Obviously the C_7-OH is *cis* to the C_3-axial OH group as dictated by the 5-membered cyclic carbonate function. Only the configuration of the C_7-Me group remains obscure.

The absolute configuration of the sugars (excluding C_7) is indicated from Hudson's isorotation rule[11] and the conformational assignments of the anomeric protons.

[11] C. S. Hudson, *J. Am. Chem. Soc.* **31**, 66 (1909); **60**, 1537 (1938).

Decarbonated methyl aldgaroside A is more positive ($[\alpha]_D$ + 73°) than decarbonated B ($[\alpha]_D$ −37°) and since the C_1-H in A has an equatorial orientation, A should be an α-D glycoside. Likewise the more negative anomer B is a β-D sugar.

We are uncertain as to which sugar occurs in the antibiotic as the IR and NMR evidence is contradictory. The IR spectrum of methyl aldgaroside A contains the 1800 cm^{-1} absorption and hence A would appear to be the anomer present in the antibiotic. However, the NMR spectrum of aldgamycin C clearly shows the anomeric proton signals for mycinose and aldgarose as one-proton doublets at 4·50 and 4·60 δ with coupling constants of 8·0 c/s each (Fig. 6), which suggests that B is the naturally occurring sugar. The same conclusion can also be obtained from the spectrum of aldgamycin E but in this case the signals are not as clear as in C. This discrepancy may be the result of some subtle stereochemical or electronic effects imposed upon the sugar by the aglycone portion of the antibiotic.

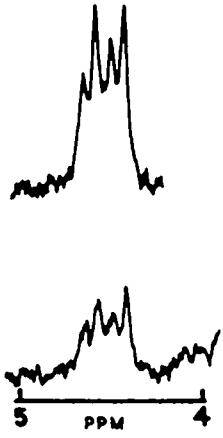


FIG. 6 The δ4 to 5 region of the NMR spectrum of aldgamycin C.

The mass spectrum of B (Fig. 7) is consistent with structure IV although it was equally in accord with II and hence is not definitive in this respect. The molecular ion is observed at *m/e* 232 with significant peaks at *m/e* 190, 172, 130 and the base peak at 86. Loss of propene to give *m/e* 190 followed by loss of methyl formate to give *m/e* 130 or the reverse with loss of methyl formate to *m/e* 172 then loss of propene to give *m/e* 130 appear to be logical fragmentation pathways. The *m/e* 130 species could then lose CO_2 or C_2H_4O to give the base peak at *m/e* 86. Several of these fragmentations are substantiated by the occurrence of metastable ions. In addition, it seems likely that equilibration of the carbonate function between the C_7-C_3 position and C_3-C_2 could occur during ionization and support of this possibility is found in the origin of the *m/e* 110 ion by apparent loss of H_2CO_3 from *m/e* 172 along with the appearance of the appropriate metastable ion.

The loss of the anomeric MeO group is represented by the ion at 201 although this appears to be a minor fragmentation.

Aldgarose represents the first cyclic carbonate sugar to be isolated from nature and

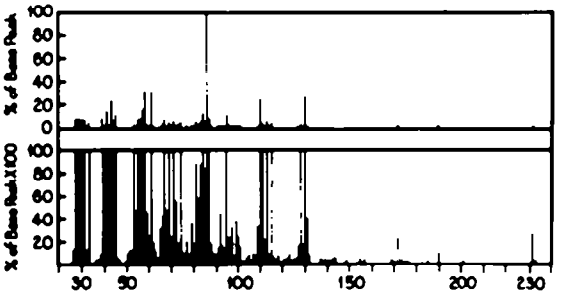
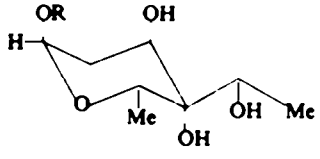


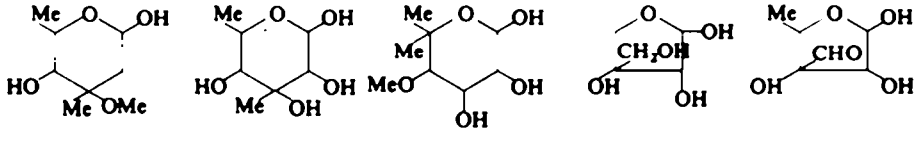
FIG. 7. Mass spectrum of methyl aldgaroside B. (Atlas CH4, Direct Inlet).

is most probably biogenetically related to the branched chain octose (V) from isoquinocycline A.[12] Grisebach has recently classified branched chain sugars into two groups: those with branched methyl derived from methionine, such as cladinose



V

(VI), mycarose (VII) and noviose (VIII), and those with branched methylol and formyl groups, represented by apiose (IX) and streptose (X) formed by rearrangement of a straight chained C_6 sugar.[13] Aldgarose and (V) are apparently the first two representatives of a third class of presently unknown biogenetic origin.



VI VII VIII IX X

EXPERIMENTAL

The IR spectra (KBr discs) were taken on a Perkin Elmer model 137 infracord. NMR spectra were recorded with a Varian A-60 in $CDCl_3$; shifts are expressed as δ values (parts per million) from TMS as internal standard and coupling constants (J) are expressed in c s. The spin-decoupling work was done on a Varian DP60 equipped with a Varian Integrator-Decoupler. M.ps are uncorrected. The mass spectra

[12] A. Tulinsky, *J. Am. Chem. Soc.* **86**, 5368 (1964); J. S. Webb, R. W. Broschard, D. B. Cosulich, J. H. Mowat and J. E. Lancaster, *Ibid.* **84**, 3183 (1962).

[13] H. Grisebach. Presented in a lecture at the Institute of Microbiology, Rutgers University on October 20, 1966.

were obtained on a direct inlet Atlas CH4. Microanalyses were performed by the Microanalytical Department of the Organic Chemical Research Section of these Laboratories.

Treatment of aldgamycin E with acid and base. Aldgamycin E (100 mg) was dissolved in MeOH (1 ml) and added to 5 ml 0·1N HCl. This soln was heated on a steam bath for 2 hr, and then the solvent evaporated under reduced press. The IR spectrum of the resulting glass still contained the 1800 cm^{-1} band, although it appeared somewhat broader toward lower frequencies.

The glass was redissolved in MeOH (1 ml) and added to 5 ml 0·1N NaOH. This was heated on a steam bath for 2 hr during which time the soln slowly darkened. This was cooled and acidified with some 5N HCl (soln bubbled) and then evaporated under reduced press to a residue. Trituration with $CHCl_3$ and evaporation under reduced press gave a brownish residue. The IR spectrum was changed from that of aldgamycin E and in particular no longer contained the 1800 cm^{-1} carbonyl band.

Conversion of aldgamycin E to aldgamycin C. Aldgamycin E (1·0 g) was dissolved in MeOH (20 ml). To this was added 100 ml 0·05N $Ba(OH)_2$ and the mixture stirred under an atmosphere of N_2 for 45 min. This was then filtered to give 228 mg $BaCO_3$ (86% of theoretical). The filtrate was extracted with three 100-ml portions $CHCl_3$ and the $CHCl_3$ extract dried with Na_2SO_4, filtered, and evaporated under reduced press to give 970 mg of a foam. The foam was dissolved in ether, filtered through a norite-diatomaceous earth pad and concentrated to a small volume whereupon crystals formed. The soln was allowed to further crystallize overnight, and then filtered to give 700 mg of plates, m.p. 150–153°. A mixture m.p. with an authentic sample of *aldgamycin C* (m.p. 149–152°) isolated from culture AL471 (see below) was undepressed. An analytical sample was prepared by recrystallization from CH_2Cl_2–ether followed by drying of the crystals at 100° 10^{-3} mm for 7 days. (Found: C, 60·62; H, 8·50; O, 31·14; O-methyl, 4·65; C-methyl, 11·06; M.W., 844 ($CHCl_3$). $C_{36}H_{60}O_{14}$ (716·9) requires: C, 60·31; H, 8·44; O, 31·25; O-methyl, 4·19 (2); C-methyl, 10·49 (5), 12·58 (6).) $[\alpha]_D^{25}$ −70° ± 4·5° (*c* = 0·670 MeOH). The UV spectrum (MeOH) showed a max at 217 mμ ($E_{1cm}^{1\%}$ = 190). The IR spectrum was identical with that of aldgamycin C isolated from culture AL471.

Isolation of algamycin C from culture AL471. Aldgamycin C was isolated from culture AL471 concentrates by further work-up of the bioactive fractions from the original alumina chromatographies which did not contain aldgamycin E.[2] These were combined (from several columns) and rechromatographed on silica gel, eluting with AcOEt and MeOH AcOEt mixtures. The material from the bioactive fractions was chromatographed on a diatomaceous earth partition column using the system n-hexane (b.p. 60–68°) AcOEt, MeOH, water (50:50:12:8). The bioactive material from this column was rechromatographed on silica gel using a linear gradient between AcOEt and 25% MeOH-in-AcOEt. The bioactive eluate was chromatographed on a diatomaceous earth partition column using the system cyclohexane, AcOEt, MeOH, water (1530:1170:351:189). The second main band of bioactive material was rechromatographed on another diatomaceous earth partition column using the same system. This afforded 113 mg of a glass which slowly crystallized on standing. Recrystallization from ether gave *aldgamycin C*, m.p. 148–152°.

Methanolysis of aldgamycin E. Aldgamycin E (10·0 g) was dissolved in MeOH (20 ml) and 10 ml of 1:1 MeOH conc H_2SO_4 was added. This was left at room temp overnight during which time the soln turned dark red-brown. This was diluted with 100 ml water and extracted with three 75 ml portions $CHCl_3$. The combined $CHCl_3$ extracts were dried with Na_2SO_4, filtered, and then evaporated under reduced press to a residue (930 mg). This was dissolved in $CHCl_3$ and chromatographed on 100 g silica gel and the sugars eluted with $CHCl_3$ through 30% AcOEt–$CHCl_3$. Very little separation was observed among the sugars. The fractions containing the sugars were combined to give 388 mg of an oil which was heated at 100° (atmos press) in the presence of a cold finger, during which time 49 mg of a crystalline methyl glycoside, m.p. 83–84°, sublimed out of the oil. This was subsequently shown to be identical with methyl mycinoside (IR spectrum, m.p., mixture m.p.). The residue was then heated at 125° (10^{-3} mm) in the presence of a cold finger to give 90 mg of an oily distillate. Treatment of this distillate with CH_2Cl_2–ether afforded 36 mg of crude *methyl aldgaroside B*, m.p. 149–153°. The residue from the mother liquor of this crystallization afforded 5 mg of crude *methyl aldgaroside A*, m.p. 91–94°, when treated with ether–hexane.

Subsequent column eluates contained some partially methanolyzed material. These fractions were combined and refluxed for an hour in methanolic H_2SO_4 which afforded an additional 72 mg of methyl aldgaroside B and an additional 34 mg of methyl aldgaroside A.

An analytical sample of methyl aldgaroside B, m.p. 175–177°, was prepared by recrystallization from CH_2Cl_2–ether. $[\alpha]_D^{25}$ = −41° ± 3° (*c* = 1·004 MeOH). (Found: C, 51·49; H, 7·05; O-methyl, 7·89; C-methyl, 6·09; M.W., 254 ($CHCl_3$). $C_{10}H_{16}O_6$ (232·2) requires: C, 51·73; H, 6·95; O-methyl, 6·74 (1); C-methyl, 12·95 (2).)

Decarbonation and periodate oxidation of methyl aldgaroside B. Methyl aldgaroside B (25 mg, 0·108 mmole) was dissolved in MeOH (1 ml) and 5 ml water. 0·32N $Ba(OH)_2$ (1 ml) was added, and the resulting mixture left at room temp for 1 hr. CO_2 was then bubbled through the mixture followed by filtration and the filtrate concentrated under reduced press to a residue. This was triturated with ether and the ethereal soln concentrated to give 22 mg (0·107 mmole) of *decarbonated methyl aldgaroside B.*

The decarbonated methyl aldgaroside was dissolved in 10 ml 0·1M phosphate buffer (pH 6·0) to which was added 48 mg $NaIO_4$ (0·224 mmole). N_2 was bubbled overnight through this soln and into a 2,4-dinitrophenylhydrazine trap. During this time a ppt formed in the trap which was filtered, washed with water and dried to give 24 mg (0·107 mmole) acetaldehyde-2,4-dinitrophenylhydrazone (identified by IR spectrum, m.p., and mixture m.p. with an authentic sample).

The aqueous periodate soln was acidified to pH 2 with H_2SO_4 and then continuously extracted with ether overnight. The ethereal extract, on workup, gave 16 mg of an oily residue. This was treated with 5 ml of 1·0N HCl and the resulting soln heated on the steam bath for 4 hr. Addition of a 2,4-dinitrophenylhydrazine stock soln, resulted in an immediate orange ppt. This was filtered, washed with water, and dried to give 40 mg (0·096 mmole) glyoxal-bis-2,4-dinitrophenylhydrazone (identified by IR spectrum, m.p., and mixture m.p. with an authentic sample).

Decarbonated methyl aldgarosides A and B. Methyl aldgaroside B (100 mg) in 25 ml MeOH was treated with 5 ml of sat. $Ba(OH)_2$ aq. After standing at room temp for 1 hr, the soln was neutralized with CO_2, filtered, and concentrated to dryness to give a colourless gum. Chromatography over acid washed silica gel by elution with AcOEt–$CHCl_3$ (4 : 1) gave 76 mg of *decarbonated B* as a colourless gum. This material showed only one spot by TLC (H_2SO_4 charring, R_f 0·36 on silica gel G plates in AcOEt); $[\alpha]_D^{25}$ −36·8° ± 2·52° (*c* = 1·19 $CHCl_3$). Methyl aldgaroside A was decarbonated and purified in the same manner as described for B. Upon TLC with the same conditions as above, *decarbonated A* showed one spot at R_f 0·33 $[\alpha]_D^{25}$ +73° ± 10° (*c* = 0·3 $CHCl_3$).

Diacetate of decarbonated methyl aldgaroside B. Decarbonated methyl aldgaroside B (60 mg) was left in Ac_2O (0·5 ml) and pyridine (0·5 ml) overnight at room temp. The soln was then evaporated and the pale yellow gum chromatographed over acid washed silica gel. Elution with benzene–AcOEt (1 : 1) provided the *diacetate* as a colourless gum (55 mg). TLC on silica gel G with benzene–AcOEt (1 : 1) showed only one spot (R_f 0·55).