spectrum (ν, cm^{-1}): 1650-1660 w, 1600-1510 s, 1470 m, 1420 w, 1375 w, 1340, 1320 m, 1240-1260 vs, 1185, 1125 s, 1050-1090 vs, 970 s, 880, 850, 770 m, 740 w. Found: N 7.65; P 8.89%. $C_{16}H_{27}N_2O_6P$. Calculated: N 7.49; P 8.32%.

Action of CH_3I on Betaine (IV). A mixtutre of (IV) in abs. CH_2Cl_2 with excess CH_3I was kept in a dry box at ~20°C for 3 days. The solvent was removed under vacuum and the residue treated with ether to give a pale-yellow powder with a mp of 175-177°C. IR spectrum (ν, cm^{-1}): 1660 m, 1600 s, 1565 m, 1520 s, 1470, 1420, 1370 m, 1270 vs, 1240 s, 1185 s, 1030-1070 vs, 970, 850, 740 s. Found: N 6.31; P 7.19%. $C_{16}H_{25}N_2O_5PI$. Calculated: N 5.80; P 6.42%.

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CONCLUSIONS

Tri(dimethylamino)phosphine and trimethyl phosphite react with the 2-p-dimethylamino-anil of 1,3-dimethyl-1,2,3-propanetrione to form a bipolar 1:1 adduct with a P–N bond.

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MYCOBACTERIUM POLYSACCHARIDES.

1*. 4-O-[(S)-1'-CARBOXYETHYL]-D-MANNOSE ISOLATED FROM THE EXTRACELLULAR POLYSACCHARIDE OF MYCOBACTERIUM LACTICOLUM STRAIN 121

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Recently, the polysaccharides of certain Gram-negative bacteria have been found to contain new monosaccharides containing lactic acid residues bonded to hexoses by an ether linkage [1-5]. We have recently [6] isolated a new acid, viz., 4-O-(1'-carboxyethyl)mannose (I) from the extracellular polysaccharide of M. lacticolum strain 121. In the present article, we will present data on the absolute configuration of (I) and its synthesis.

Acid (I) was isolated from the polysaccharide hydrolysate by ion-exchange chromatography and preparative paper chromatography (PC). Methanolysis of (I) followed by ammonolysis and treatment of the resulting amide (II) with NaOCl [7] gives a methyl mannoside (III) with an $[\alpha]_D$ value of +66.8°, and hydrolysis of this gives mannose, which was identified by comparison with an authentic sample using PC, ion-exchange chromatography, and GLC (in polyacetate form)

*See [6] for preliminary communication.

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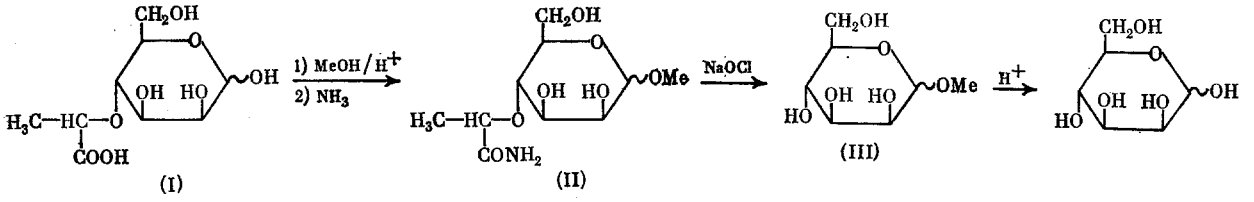
TABLE 1. Specific Rotations of 4-O-[1'-carboxyethyl]-D-mannoses (I), (IXa), and (IXb)

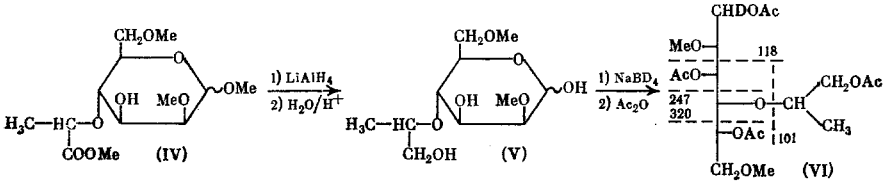
Compound	$[\alpha]_D$ (C, water)
(I)	+15,05° (2)
(IXa)	+14,3° (5,9)
(IXb)	+57,3° (3,9)



(I) (II) (III)

Bearing in mind that methyl-α-D-mannopyranoside has an $[\alpha]_D$ value of +79.2° and the β isomer has a value of −49°, and that methanolysis of D-mannose gives predominantly methyl-α-D-mannopyranoside [8], we may suppose that the mannose residue in (I) has a D configuration.

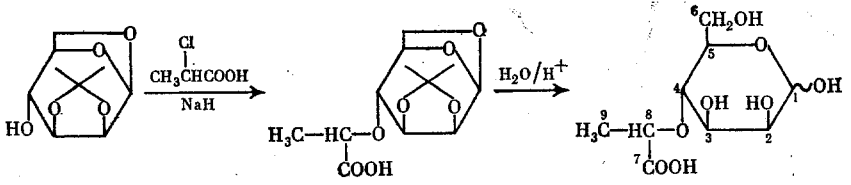
The position of the lactic acid residue in (I) was traced from the mass spectrum of the polyol (VI) obtained by successive $LiAlH_4$ reduction, hydrolysis, $NaBD_4$ reduction, and acetylation of 2,6-di-O-methyl-4-O-[1'-(carboxymethyl)ethyl]-α(β)-methylmannoside (IV), which was isolated from the methanolysate of the methylated polysaccharide by chromatography on SiO_2 [6]



(IV) (V) (VI)

The fragmentation of (VI) (see diagram) clearly shows that the new monosaccharide has a 4-O-[1'carboxyethyl]-D-mannose structure.

To determine the absolute configuration of the lactic acid residue in (I), we synthesized acids (IXa) and (IXb) from 1,6-anhydro-2,3,O-isopropylidene-β-D-mannopyranoside (VII) [9,10] and the S and R forms of α-chloropropionic acid [11]



(VII) (VIIIa, b) (IXa, b)

1,6-Anhydro-2,3-O-isopropylidene-β-D-mannopyranoside (VII) [9] was treated with the S and R forms of α-chloropropionic acid [11] in dioxane in the presence of NaH [12], giving 10-15% yields of compounds (VIIIa) and (VIIIb). Acid hydrolysis of these derivatives gives compounds (IXa) and (IXb), which were purified by chromatography on the anion-exchange resin Amberlite CG-400 (HCO_3^- form). The specific rotations of these compounds are given in Table 1. Comparison of the specific rotations of the synthetic samples of (IXa) and (IXb) with that of the natural compound (I) shows unequivocally that the lactic acid residue in 4-O-(1'-carboxyethyl)-D-mannose (I) has the S configuration.

It would be of interest to compare the ^{13}C NMR spectra of the two isomers (IXa) and (IXb) with the object of developing a method for identifying these compounds, since they

TABLE 2. Assignment of Signals from Carbon Atoms in the ^{13}C NMR Spectra of 4-O-(1'-Carboxyethyl)-D-mannoses (I), (IXa), and (IXb)

Compound	$C^1\alpha,\beta$	$C^4\alpha,\beta$; $C^5\beta$; $C^8\alpha,\beta$	$C^3\beta$	$C^2\alpha,\beta$; $C^3\alpha$; $C^5\alpha$	$C^6\alpha,\beta$	$C^9\alpha,\beta$	$C^7\alpha,\beta$
			pH 7				
(IXa), (I)	94,5	76,35; 76,0	73,8	72,2; 70,8; 70,2	60,8	19,5	181,6
(IX b)	94,5	75,3; 75,1; 74,6	73,8	72,3; 71,8; 71,7; 71,5	61,7	19,5	181,7
			pH 1				
(IXa), (I)	94,6	77,3; 76,8; 76,5; 75,7	73,7	72,6; 71,8; 71,4; 70,9	61,35	19,2	177,7
(IX b)	94,45	76,4; 76,2; 75,8; 75,5	73,9	72,3; 71,8; 71,1	61,7	18,9	177,8

obviously occur widely in nature [in particular, we have detected (I) in several other microbacterial polysaccharides]. By comparing the ^{13}C NMR spectra of compounds (IXa) and (IXb) on the one hand, and the spectra of 4-O-methyl-D-mannopyranosides (α and β) [13] and 3-methoxypropionic acid (X) on the other, we can assign the groups of signals from the C atoms of compounds (IXa) and (IXb) as shown in Table 2. Comparison of the ^{13}C NMR spectra of (IXa), (IXb), and (I) with each other (recorded at the same pH) reveals that the spectra of compounds (I) and (IXb) are different and those of (IXa) and (I) are completely identical. As can be seen from Table 2, the spectra of (IXa) and (IXb) differ most in external form at pH 7. Thus, all three lines in the $C^4\alpha$, β, $C^5\beta$, and $C^8\alpha$, β resonance region in the spectrum of (IXb) lie at higher field than the two lines assigned to the same carbon atoms in (IXa) or (I). The spectra of (IXa) and (I) also have a characteristic line at 70.2 ppm, which is not observed in the spectrum of (IXb). Consequently, the external form of the spectra recorded at pH 7, and also the presence of lines with a characteristic shift, makes it possible to identify reliably 4-O-(1'-carboxyethyl)-D-mannoses with different configurations of the lactic acid residue.

Thus, we can ascribe the 4-O-[(S)-1'-carboxyethyl]-D-mannose structure to the acidic monosaccharide isolated from the extracellular polysaccharide of M. lacticolum strain 121 on the basis of the data presented above.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded at 35°C with a WP-60 instrument at a frequency of 15.08 MHz, using D_2O solutions with DMSO as internal standard, the shift of which was taken to be 39.445 ppm (established in a special experiment); pulse width 3μsec (30°C); repetition frequency 1.1 sec; resonance stabilization by deuterium nuclei in solvent. The specific rotations were determined with a Perkin–Elmer 141-M instrument at a wavelength of 589 nm. The mass spectra were recorded using a Varian MAT Gnom III instrument with a column containing 5% SE-30.

Isolation of Acid (I). A 0.6 g portion of the polysaccharide was hydrolyzed with 2 N H_2SO_4 (100°C, 8 h). The hydrolysate was contacted with Amberlite CG-400 (HCO_3^- form) until neutral. The anion exchanger was washed with water until neutral saccharides were absent (phenol–H_2SO_4), and then with 20% CH_3COOH. The mixture of acid monosaccharides was separated on FN-8 paper with n-butanol/pyridine/water (6:4:3) or pyridine/ethyl acetate/acetic acid/water (5:5:1:3). The yield of (I) was 0.52 g, $[\alpha]_D^{20}$ = +15.05° (c = 2, water).

Conversion of Acid (I) to Mannoside (III). A 0.04 g sample of (I) was heated in dry methanol containing 2% HCl (100°C, 4 h, in an ampul). The solution was neutralized with NH_3 and evaporated to dryness. The residue was dissolved in abs. methanol and treated with dry HN_3. The mixture was kept at 20°C for 24 h and evaporated. The residue was treated with a 10% solution of NaOCl at 20°C for 4 h [7], treated with 0.2 g $NaBH_4$, neutralized with CH_3COOH, and evaporated to dryness. The residue was acetylated with Ac_2O in pyridine. The methyl-α,β-D-mannopyranoside acetates (III) were extracted with chloroform. The solution was dried and evaporated to dryness, $[\alpha]_D^{20}$ = +34.0° (c = 2.3, chloroform). Deacetylation was effected with CH_3ONa in CH_3OH [14]. The residue was passed through a column of Amberlite CG-400 (HCO^{3-} form). Elution with water gave methyl-α,β-D-mannopyranoside (III), which was identical to an authentic sample according to PC. Yield 0.022 g, $[\alpha]_D^{20}$ = 66.8° (C = 2.0, water).

A 0.005 g sample of (III) was heated in 2 ml of 0.5 N HCl at 100°C for 3 h. The solution was evaporated over P_2O_5 under vacuum. Mannose was identified in the mixture by PC and ion-exchange chromatography using a Technicon SC-2 instrument. Part of the hydrolysate was reduced with $NaBH_4$, worked up, and acetylated with Ac_2O–pyridine (1:1). Mannitol hexaacetate was identified in the mixture by GLC (column containing 3% ECNSS, 1 m, 190°C).

Determination of the Structure of Monosaccharide (IV). The polysaccharide (0.2 g) was methylated by Hakomori's method [15], subjected to methanolysis and chromatographed on SiO_2 to give 0.03 g of glycoside (IV). This was reduced with $LiAlH_4$ in ether (reflux, 6 h), hydrolyzed with 0.5 N HCl (3 h, 100°C), and reduced with $NaBD_4$, after which the mixture was acetylated with Ac_2O–pyridine (1:1). Mass spectrum of polyol (VI), m/e (%): 320 (43), 247 (38), 187 (20), 160 (15), 158 (13), 154 (11), 147 (15), 129 (40), 126 (29), 118 (95), 102 (30), 101 (100), 87 (33), 59 (34), 45 (43), 43 (90).

1,6-Anhydro-2,3-O-isopropylidene-4-O-(1'-carboxyethyl)-β-D-mannopyranosides (VIIIa,b). These compounds were synthesized by the reaction scheme proposed in [12]. A solution of 1 g (0.005 mole) of 1,6-anhydro-2,3-isopropylidene-β-D-mannopyranoside (VII) (prepared by seven-step synthesis from levoglucosan [9,10]) in 70 ml abs. dioxane was stirred vigorously while adding 0.58 g NaH (50% oil emulsion), stirred at 95°C for 1 h, cooled to 65°C, and treated with 1.4 g (0.06 mole) of (S)- or (R)-chloropropionic acid (the (S)- and (R)-chloropropionic acids were prepared by nitrosation of the corresponding alanines [11]). The mixture was stirred for 1 h, treated with 2.3 g NaH and 30 ml abs. dioxane, and stirred for 14 h at 65°C. The mixture was cooled and carefully treated with 100 ml water to decompose the excess NaH. The dioxane was distilled off in vacuo and the aqueous solution extracted with chloroform to remove the mineral oil and unreacted (VII).* The aqueous solution was adjusted to pH 3 with 2.5 N HCl at 0°C and extracted with chloroform (3 × 100 ml). The extract was washed with water, dried over Na_2SO_4, and evaporated. The residue was separated on a column of SiO_2, to give 0.11 g (11%) of (VIIIa), $[\alpha]_D^{20}$ = –11.5° (C = 2, chloroform), and 0.15 g (15%) of (VIIIb), $[\alpha]_D^{20}$ = +10.6° (C = 1, chloroform).

4-(1'-Carboxyethyl)-D-mannose (IXa,b). A mixture of 0.2 g (0.001 M) of (VIIIa,b) and 10 ml of 3% HCl was boiled for 4 h, cooled, and neutralized with Amberlite CG-400 (HCO_3^- form). The resin was washed with water and then with 20% CH_3COOH. The acid eluate was evaporated to dryness and the residue dissolved in water and treated with KU-2 (H^+ form), followed by filtration and evaporation to dryness. The products (IXa,b) were homogeneous according to PC with the above solvents. The yield of (IXa,b) was 0.161 g (82%). The specific rotations are given in Table 1 and the ^{13}C NMR spectra in Table 2.

(S,R)-2-Methoxypropionic Acid (X). A solution of 1 g (0.01 mole) of (S,R)-chloropropionic acid in 10 ml abs. methanol was treated with 0.7 g Na and heated in an ampul at 100°C for 5 h. The solution was evaporated and the residue dissolved in water and treated with cation-exchange resin KU-2 (H^+ form). The resin was filtered off and the filtrate evaporated to give 0.84 g (84%) of (X). ^{13}C NMR spectrum (δ, ppm) in D_2O (pH 7): C^1 179.7; C^2 77.94; C^3 18.44; OCH_3 57.3; (pH 1): C^1 177.3; C^2 76.54; C^3 18.13; OCH_3 57.7.

CONCLUSIONS

A new acid monosaccharide, viz., 4-O-[(S)-1'-carboxyethyl]-D-mannose, has been isolated from the extracellular polysaccharide produced by M. lacticolum, and its structure demonstrated by back-synthesis and by comparison of its ^{13}C NMR spectrum with those of authentic samples in which the lactic acid residue has an (S) and (R) configuration.

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*The starting mannoside (VII) was isolated in an amount of 0.7 g (70%) from this fraction by column chromatography on SiO_2.

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