the trinuclear complexes are formulated with a trimeric cation in which two carboxylate groups bridge each of the sides of the equilateral triangle, whose vertices are the metal atoms and whose centre is an oxygen atom.[3-5]

While the OH-groups of the aliphatic ligands do not participate directly in the complex formation, it is most likely that the phenolic OH-groups as found in the salicylic acid and the fulvic acid play an active role in this respect. Thus, methylation of the OH-groups of salicylic acid caused ΔE_α to decrease to a value similar to that of benzoic acid. Also, for a fulvic acid preparation Schnitzer and Skinner [9] found that blocking of either carboxyls or phenolic hydroxyls caused significant reductions in metal retention; and both groups appeared to react simultaneously.

Furthermore, the Mössbauer spectrum of the polynuclear Fe—FA complex indicates that the complex formation is associated with specific structures which form part of a well organized molecular system. This is in accordance with recent ESR measurements of a series of fulvic acid fractions which gave a linear relationship between $\overline{M}_n$ and free radical content per $\overline{M}_n$.[10]

Schnitzer and Hansen [11] previously found that in aqueous solutions at low pH-values, the Fe—FA complexes formed were mononuclear. For a series of metal ions it was, however, similarly demonstrated that except at high ionic strengths metal/FA ratios generally increased above 1.0 with increase in pH, indicating the formation of polynuclear complexes.

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Circular Dichroism of 2-*O*-Acetyl-3,6-dideoxy-D-*xylo*-hexopyranosides and of *Salmonella typhimurium* Lipopolysaccharides

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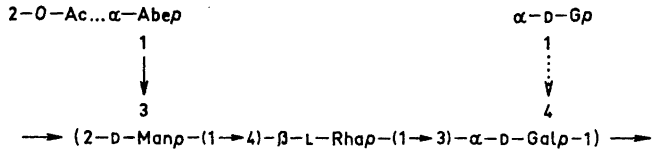
The repeating unit of the O-specific sidechains of the *Salmonella typhimurium* (serogroup B) lipopolysaccharide may be formulated as shown on top of p. 3085.[1-3]

The position of the *O*-acetyl group has been established [2,3] by methylation of a modified lipopolysaccharide in which all free hydroxyl groups had been protected by acetal formation with methyl vinyl ether.[4] After replacement of acetyl by methyl followed by acid hydrolysis the only methylated sugar present was 3,6-dideoxy-2-*O*-methyl-D-*xylo*-hexose (2-*O*-methylabequose) showing that the acetyl groups are exclusively attached to the position 2 of the dideoxy sugar. The degree of acetylation at the 2-position varies from 50 % (*S. typhimurium* LT 2) to nearly 100 % (*S. typhimurium* 395 MS). The anomeric configuration of the terminal abequose residues has been allocated on the basis of the rapid initial decrease in optical rotation of the polysaccharide in acid solution. Since 3,6-dideoxyhexosides are known to undergo fast hydrolysis in acid, the configuration at C-1 of the abequose units was thus designated as α.[2,3]



Optical rotatory dispersion (ORD) and circular dichroism (CD) provide useful methods for the determination of the configuration at asymmetric centers adjacent to a suitable chromophore. Thus, it is possible to allocate the anomeric configuration of phenyl glycosides by observing the sign of the Cotton effect in their ORD spectra.[5] Extensive studies on 2-deoxy-2-acetamido sugars, including oligosaccharides, have shown that a correlation exists between the anomeric configuration [6] and the ORD and CD spectra, respectively.

In the abequose residues in the serogroup B lipopolysaccharides, the acetoxy group is situated on a carbon atom adjacent to the anomeric carbon. It is flanked on the other side by a symmetrical methylene carbon. The sign of the dichroism observed in the CD spectrum of the polysaccharide should therefore be influenced only by the anomeric configuration at C-1; this should provide an alternative method for determining the anomeric configuration of the abequose units.

The CD spectra of methyl 2-*O*-acetyl-α and β-abequosides, the synthesis of which has been described in a previous communication,[7] are shown in Fig. 1. The α-anomer shows a negative Cotton effect, whereas for the β-anomer, an effect of the same magnitude but of opposite sign is observed. The CD spectrum of the delipidated polysaccharide [8] from *Salmonella typhimurium* 395 MS is shown in the same figure. The sign of the Cotton effect and the position of the maximum correspond to the acetylated α-glycoside. The difference in the magnitude of the signal merely reflects the attachment of the abequose unit in the polysaccharide to a mannose unit instead of to a methyl group as in the glycoside.

The results therefore confirm that the 2-*O*-acetylabequose residues are attached by an α-linkage to the backbone of the *O*-antigenic side-chain in the *Salmonella typhimurium* lipopolysaccharide.

Many bacterial polysaccharides contain *O*-acetylated sugar residues. Although the abequose units in the present study are unique in that the chromophore is flanked by only one asymmetric carbon (C-1) and a clearcut relation between dichroism and the anomeric nature of the anomeric carbon is observed, useful relationships between CD and the position of the *O*-acetyl groups and/or the geometry at vicinal asymmetric carbons might possibly exist in other polysaccharides. This is currently under investigation.

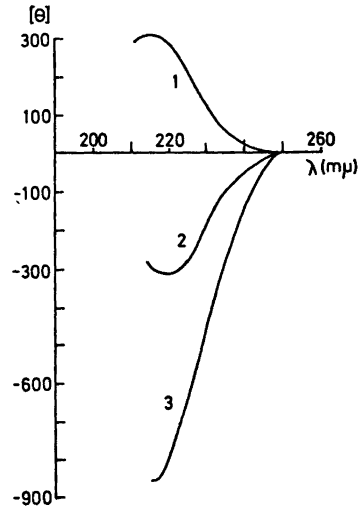


Fig. 1. CD spectra of methyl 2-*O*-acetyl-β-abequoside (1), methyl 2-*O*-acetyl-α-abequoside (2) and delipidated polysaccharide from *Salmonella typhimurium* 395 MS (3). The value for [θ] for 3 is based on the actual amount of 2-*O*-acetylabequose in the polysaccharide.

Experimental. The synthesis of the 2-*O*-acetyl-3,6-dideoxyhexosides has been described previously.[7] CD was determined on a Cary 60 apparatus equipped for CD.

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Thin Layer Chromatography (Bioautography) of Streptomycins

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A simple and rapid chromatographic method to separate and detect the components of the streptomycin group of antibiotics is of importance, as these may occur simultaneously.

Several chromatographic systems have been described,[1-4] but the resolution obtained is often poor, or the procedures are too complicated and time-consuming for routine work.

We have developed a thin layer chromatographic system, which gives a good separation of many streptomycins.

Silica gel plates were prepared by dipping glass plates measuring 5 by 20 cm in a 30 % (w/v) suspension of silica gel containing 13 % gypsum (Merck, Darmstadt, West Germany) in chloroform.

The gel layer on one side and the edge was removed. After drying in a current of air 2μ samples of solutions each containing 5 to 10 mg/ml of the compounds were applied.

The spots were dried in a current of air before development. The chromatograms were developed in distilled water for 1 h. After drying the chromatograms were either sprayed with the α-naphthol-diacetyl reagent, described by Halliday,[5] giving purple spots on a white background or bioautographed.

The bioautography was performed as follows:

Pieces of filter paper of the same size as the chromatograms were dipped in 0.1 N HCl and applied to the chromatograms. The wet papers were pressed to the silica gel layer by means of 1 cm thick glass plates for 20 min, and then allowed to dry while still placed on the chromatograms. To remove the last traces of HCl the papers were subsequently dried for 1 h in an oven at 40°C. The dried papers were applied to agar plates seeded with spores of *Bacillus subtilis* ATCC 6633 for 1—5 min, depending on the amounts and activities of the antibiotics. The agar plates were incubated for 16 h at 37°C yielding clear zones of inhibition. R_F values for some streptomycins are given in Table 1.

Table 1. R_F values relative to streptomycin.

Streptomycin	1.00
Streptidine	1.55
Dihydrostreptomycin	0.90
Dihydro-*N*-demethylstreptomycin	1.16
Hydroxystreptomycin	1.22
Streptomycylamin	0.53
Dihydrodesoxystreptomycin	0.80
Methylstreptomycin	0.66

The absolute R_F values varied considerably from run to run. The R_F value for streptomycin was in most cases between