Structural Studies of the Capsular Antigen from *Hæmophilus influenzæ* Type b

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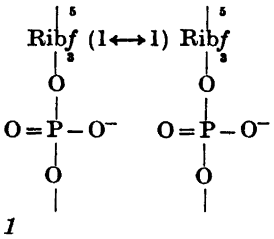
The structure (*1*) proposed by Zamenhof *et al.*[1] for the capsular antigen from *Hæmophilus influenzæ*, type b, is at variance with established principles for the biosynthesis of teichoic acids and related polymers.[2] The structure must therefore be incorrect or the polymer is synthesized by a route, differing from those previously outlined. In order to resolve this matter, a reinvestigation of the structure was undertaken. During the course of this investigation, Crisel *et al.*[3] showed that the structure was, indeed incorrect and demonstrated that the polymer is composed of repeating units having the structure *2*. We now report some results from our own studies, which support this structure and give further structural details.



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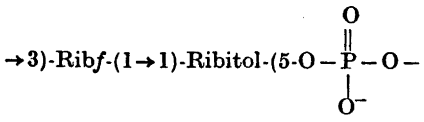

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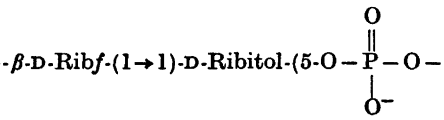
In agreement with Crisel *et al.* we find that the polymer is composed of equimolecular parts of D-ribose, ribitol and phosphate. On dephosphorylation, by treatment first with alkali and then with phosphatase, a ribosylribitol is produced. Methylation analysis [4] of this disaccharide gives a mixture of 2,3,5-tri-*O*-methyl-D-ribose and 1,2,3,4-tetra-*O*-methylribitol, demonstrating that the disaccharide is a 1-*O*-D-ribofuranosylribitol. The MS of the fully methylated disaccharide [5] is in agreement with this proposal. The optical rotation of the disaccharide, $[\alpha]_{589}^{25}$ −28°, indicates that it is a β-D-ribofuranoside.

We recently prepared 1-*O*-β-D-galactopyranosyl-D-ribitol and the corresponding L-ribitol derivative.[6] The optical rotation of the former showed a large negative shift in molybdate at pH 5.5[7] and a corresponding positive shift was observed for the L-isomer. Since it has been established that molybdate complexes with the ribitol moiety and not the glycosyl moiety,[8] we may conclude that the shifts are typical for 1-*O*-substituted D- and L-ribitol derivatives, respectively. The 1-*O*-β-D-ribofuranosylribitol from *Hæmophilus influenzæ* type b shows $[\alpha]_{589}^{25}$ −80°, $[\alpha]_{578}^{25}$ −83°, $[\alpha]_{546}^{25}$ −102°, $[\alpha]_{436}^{25}$ −204° and $[\alpha]_{365}^{25}$ −417° in sodium molybdate buffered to pH 5.5, demonstrating that the ribitol moiety has the D-configuration. The phosphate, linked to the other primary position in the ribitol moiety,[3] is consequently linked to O−5 in D-ribitol. This is in agreement with the postulated biosynthetic route [2] in which ribitol phosphate most probably derives from cytidine diphosphate ribitol, in which D-ribitol is phosphorylated at O−5. The previous results on the structure of the capsular antigen from *Hæmophilus influenzæ* type b are therefore supplemented and a complete structure of the repeating unit *3* is proposed.



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Experimental. Solutions were concentrated under reduced pressure at bath temperatures not exceeding 40 °C. For GLC, a Perkin-Elmer 990 instrument fitted with flame ionisation detectors was used. Separations were performed on columns of 3 % OV-1 on Gas-Chrom Q and 3 % ECNSS-M on Gas-Chrom Q. For GLC-MS, a Varian Mat-311-SS 100, MS computer system was used. Preparative PC was performed on Whatman No. 1 paper, employing butanol−pyridine−water, 6:4:3, as the solvent system. Alkaline silver nitrate was used for detection.

Capsular antigen (60 mg) from *Hæmophilus influenzæ* type b, strain RAB, prepared as previously described,[9] was treated with 0.5 M aqueous sodium hydroxide (10 ml) at 100 °C for 4 h and worked up as described by Armstrong *et al.*[10] The product in sodium hydrogen carbonate buffer of pH 10.4 (10 ml) was treated with alkaline phosphatase (10 mg, Sigma Chemical Company) for 3 days at 37 °C. The hydrolysate was fractionated on a Sephadex G-15 column (2.6 × 100 cm) irrigated with water, the separation being followed by dif-

ferential refractometry. The two components in the disaccharide region were separated by PC ($R_{ribitol}$ 0.13 and 0.86). NMR of the fast component (6 mg) showed, *inter alia*, a signal at δ 5.03, $J_{1,2}$ 1.5 Hz, assigned to the anomeric proton. The component showed $[\alpha]_{589}^{25}$ −28° (*c* 0.6, water) and $[\alpha]_{589}^{25}$ −80°, $[\alpha]_{578}^{25}$ −83°, $[\alpha]_{546}^{25}$ −102°, $[\alpha]_{436}^{25}$ −204°, and $[\alpha]_{365}^{25}$ −417° (*c* 0.2, 0.037 M sodium molybdate at pH 5.5). The oligosaccharide (2 mg) in dimethyl sulfoxide (1 ml) was treated with 2 M sodium methylsulfinyl anion in dimethyl sulfoxide (1 ml) for 4 h at room temperature and methyl iodide (1 ml) was added under external cooling with ice. The solution was diluted with chloroform (5 ml) and extracted with water (6 × 2 ml). The chloroform phase on GLC-MS gave a single peak with the MS expected [5] for fully methylated 1-*O*-β-D-ribofuranosylribitol, and showing, *inter alia*, the following ions: 45(63), 55(6), 59(23), 71(36), 74(5), 75(11), 83(5), 87(5), 88(6), 89(17), 99(7), 101(100), 102(7), 103(5), 111(6), 115(7), 133(2), 143(8), 145(3), 175(4), 177(1), 191(4) and 207(1).

The fully methylated disaccharide was hydrolysed with 0.25 M sulfuric acid (0.5 ml) at 100 °C for 16 h, reduced ($NaBH_4$), acetylated and analysed by GLC-MS. Two components, with MS corresponding to 1-*O*-acetyl-2,3,4,5-tetra-*O*-methylribitol and 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylribitol, were obtained.

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A Photolytic Study of Ethoxy(diphenylmethylene)sulfonium Ion and Hydroxy(diphenylmethylene)sulfonium Ion. Alkylation of Benzophenone, Thiobenzophenone and Thiobenzophenone-*S*-oxide

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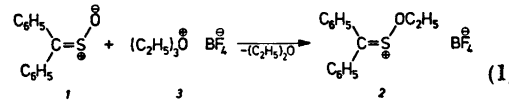
In connection with our investigations on the photolytic transformation of sulfines (thiocarbonyl-*S*-oxides) into the thermally unstable oxathiiranes,[1] we have studied the photolysis of thiobenzophenone-*S*-oxide (*1*) in concentrated sulfuric acid. In addition, with the aim of eventually obtaining more stable derivatives, we have examined the photochemistry of the hitherto unknown ethoxy(diphenylmethylene)sulfonium tetrafluoroborate (*2*).

Compound *2* is prepared by heating a mixture of *1* and triethyloxonium tetrafluoroborate in the solid state; it is analyzed as described in the experimental section. The yellow, crystalline, highly hygroscopic compound exhibits a UV absorption maximum at 369 nm ($\varepsilon = 1.77 \times 10^4$ M^{-1} cm^{-1}).



(1)

On hydrolysis of *2*, elemental sulfur is formed along with benzophenone in greater than 90 % yield. In analogy with the acid catalyzed hydrolysis of *1* reported by Strating *et al.*[2] the hydrolysis may be formulated as shown in eqn. 2.

(2)

Compound *2* may be dealkylated to the starting sulfine in 25 % yield with triphenylphosphine in methylene chloride.

When *1* was dissolved in concentrated sulfuric acid an orange solution was obtained. The absorption bands of *1* [3] were replaced by a band with maximum at 364 nm ($\varepsilon = 1.59 \times 10^4$ M^{-1} cm^{-1}), close to that of *2* indicating protonation of *1* at oxygen. Cautious addition of water (cooling) afforded *1* in quantitative yield.