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A METHOD FOR THE SULFATION OF SUGARS, EMPLOYING A STABLE, ARYL SULFATE INTERMEDIATE

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ABSTRACT

A method for the synthesis of sugar sulfates is described which, unlike the methods in general use, involves incorporation of the sulfate function in the form of a protected organosulfate. For example, the reaction of phenyl chlorosulfate with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose afforded the 3-(phenyl sulfate) of the latter in high yield. Deprotection, to obtain the 3-sulfate derivative, was readily effected by catalytic hydrogenolysis. As the (phenyl sulfate) substituent is relatively stable under a variety of conditions, it would be expected to survive an array of chemical transformations made on esterified sugars, or derivatives. Also, it is more compatible with general synthetic and purification procedures than an ionic sulfate group. For these reasons, the (phenyl sulfate), or analogous organosulfate, substituent should be particularly well suited to the synthesis of complex sulfates, including those of higher saccharides.

INTRODUCTION

Sulfate groups are prominent substituents of the glycosaminoglycans commonly known as "mucopolysaccharides", *e.g.*, heparin and the chondroitin sulfates, as well as of such seaweed polysaccharides as carrageenan and agar. Various sugar sulfates have been synthesized[1] for structural studies on these polymers, usually by a base-catalyzed reaction between an appropriately substituted derivative of the sugar and chlorosulfonic acid or sulfur trioxide.

In undertaking syntheses of sulfated oligosaccharides that are structurally related to heparin, we have examined the possibility of introducing a protected organosulfate substituent that would be more compatible than anionic sulfate with the variety of chemical transformations envisaged. This may be likened to the phosphoric triester approach[2] in oligonucleotide synthesis, which avoids disadvantages inherent in the anionic function of phosphoric diesters. In an early attempt[3] to employ a methyl sulfate substituent in this way, the methyl group proved to be too susceptible to alkyl fission for most purposes. Phenyl chlorosulfate, introduced[4] for the regioselective substitution of primary hydroxyl groups, has been used in a synthesis of D-glucose 6-sulfate. Although the latter product was obtained in low yield, the

properties described for the aryl sulfate intermediate appeared to offer good potential for our purposes. The present study has served to confirm this expectation.

RESULTS AND DISCUSSION

Synthesis of phenyl sulfate derivatives. — Typically, the sulfation of sugars is accomplished[1] with chlorosulfonic acid in pyridine, or sulfur trioxide in either pyridine or *N,N*-dimethylformamide. Although good yields are obtained, the products are often difficult to purify[1,5,6], and properties of the sulfate function may interfere[7] with subsequent reactions of the sugar derivative. Such difficulties as these should be lessened by use of a protected sulfate substituent that remains in place during manipulations at other positions in the molecule, and from which the protecting group is removed at an ideal stage:

$$M\text{-}OH \rightarrow M\text{-}O\text{-}SO_2\text{-}OR \rightarrow M'\text{-}O\text{-}SO_2\text{-}OR \rightarrow M'\text{-}O\text{-}SO_3^-,$$

where MOH = original alcohol, M′ = modified molecules, and R = a protecting group.

As already noted, the methyl group is unsuitable in a protecting role. We attempted, unsuccessfully, to prepare an isobutyl derivative (**2**) by reaction between 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**) and isobutyl chlorosulfate[11] in ether–pyridine. Moreover, there was little evidence of sulfation when phenyl chlorosulfate was added to **1** under the same conditions, or in oxolane–collidine*, and to a derivative having a primary hydroxyl group, *viz.*, 1,2:3,5-di-*O*-methylene-α-D-glucofuranose (**5**). However, after treatment with sodium hydride in freshly distilled oxolane, both **1** and **5** reacted readily with phenyl chlorosulfate, to give, respectively, a 75% yield of **3** and a 77% yield of **6**.

Chemical properties of the phenyl protecting group. — A series of experiments conducted with **3** and **6** furnished information about the stability of the phenyl group, and provided a suitable means for its removal. Mildly acidic conditions that readily hydrolyzed the 5,6-*O*-isopropylidene group of **3**, namely, use of trifluoroacetic acid in chloroform, or of a cationic resin in 1:1 water–oxolane, at room temperature, had little effect on the phenyl group**. At an elevated temperature, or in the presence of hydrochloric acid, hydrolysis of the phenyl group, as well as of the acetal groups, occurred. However, acetolysis of **3** with 1:1 acetic anhydride–acetic acid containing sulfuric acid effected the removal of both of the *O*-isopropylidene groups, but not the phenyl group; *i.e.*, the product appeared (n.m.r. evidence) to be primarily 1,2,5,6-tetra-*O*-acetyl-β-D-glucofuranose 3-(phenyl sulfate) (**7**).

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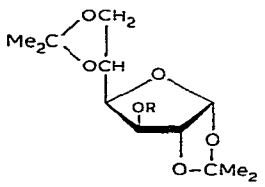
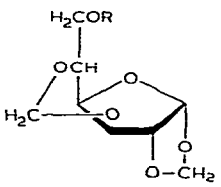
*In agreement with ref. 4, substitution did take place with *N,N*-dimethylformamide as the solvent. However, the yield of product was low, and it appeared that the halide reacted with this solvent.

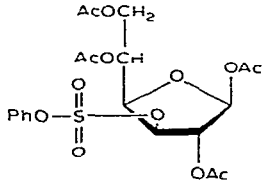
It is worth noting that a solution of **3 in 2:1 D_2O–Me_2SO-d_6 was found to produce a ^1H signal corresponding to the formation of ~25% of phenol in 27 h at room temperature. Possibly, this is related to the known[9] instability of sulfate groups in aqueous dimethyl sulfoxide.



1 R = H
2 R = $Me_2CHCH_2OSO_2$
3 R = SO_3Ph
4 R = SO_3Na



5 R = H
6 R = SO_3Ph



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At room temperature, product **6** was stable in sodium methoxide solution, and also in 2:1 ammonium hydroxide–pyridine. Although methyl phenyl sulfate decomposes[10] in sodium methoxide at 25°, it undergoes *alkyl* fission, which is less likely for the sugar moiety of **6**, because its carbon atoms should be less electrophilic. The phenyl sulfate group was also stable towards fluoride ion, which readily displaces phenoxide from phenyl phosphoric esters[11]. That is, phenol was not detected when **6** was treated with cesium fluoride in acetonitrile or methanol[11], or with potassium fluoride and 18-crown-6 (ref. 11), although an unidentified reaction occurred with tetrabutylammonium fluoride in oxolane.

These findings show that a phenyl sulfate substituent may be expected to survive a number of manipulations made on various sugar derivatives bearing this substituent. Such manipulations include selective acid-hydrolysis, acetolysis, deacetylation, and other base-catalyzed reactions, and removal of trialkylsilyl substituents with fluoride ion. Clearly, therefore, the phenyl group offers much promise as a means of protecting a sulfate group and masking its anionic character during the course of a synthetic sequence.

Removal of the phenyl protecting group. — In addition to the aforementioned advantages is the fact that the phenyl group may be readily removed by catalytic hydrogenolysis; this was demonstrated with products **3** and **6**, by using platinum oxide as the catalyst, 95% ethanol as the solvent, and potassium carbonate as the buffer. It is noteworthy that the phenyl group *per se* was not removed, but was first hydrogenated to a cyclohexyl group. The latter was labile, in common with other alkyl groups, and appeared as cyclohexanol in the hydrogenolysis reaction-mixture*. Therefore, alkyl fission was ultimately responsible for removal of the phenyl group, and it occurred with high selectivity, to afford the sugar sulfate, rather than in the direction of desulfation. The possibility that ~10% of desulfation had taken place was suggested by the presence of a weak, aryl ^1H signal in the ^1H-n.m.r. spectra of the products from both **3** and **6**; this signal was consistent with the presence of phenyl sulfate, but not of phenol. The impurity was removed with an anion-exchange resin,

*By contrast, in the hydrogenolysis of phenyl phosphoric esters[12,13], cyclohexane is liberated.

from which the sugar sulfate was selectively eluted with ammonium hydrogencarbonate, and then converted into the sodium salt (*e.g.*, **4**).

Preparation and properties of phenyl chlorosulfate. — Phenyl chlorosulfate has been prepared[4] in moderate yield by the reaction of phenol with sodium, followed by treatment with sulfuryl chloride. In attempting to improve the method of preparation, the sodium metal was replaced by pyridine, triethylamine, or sodium hydride. However, these changes led to chlorination of the phenol to the extent of 80, 50, and 20%, respectively. A more satisfactory method of preparation consisted of allowing phenol to react with sodium hydroxide, and treating the sodium phenoxide[14] in benzene* with sulfuryl chloride; this gave a product of >80% purity, which was further purified by distillation. The distillate was a colorless liquid (d = 1.39), stable indefinitely at <0°, and showing absorption in the i.r. region, at 1410 and 1195 cm^{-1}, characteristic[15] for $-SO_2Cl$, as well as a resonance signal at δ 7.3 in its ^{1}H-n.m.r. spectrum, due to the phenyl protons. Phenyl chlorosulfate has a very lingering odor, and, although it is not so strong a lachrymator as alkyl chlorosulfates, and no adverse physiological effects were observed during its use, the compound should be treated with caution, pending toxicological testing.

In summary, the properties of the sulfating agent, and of the phenyl protecting group that it introduces, satisfy, in many respects, the requirements stated at the outset. Synthesis of oligosaccharide sulfates may be contemplated, whereby the phenyl sulfate substituent is placed on an appropriately protected monosaccharide; the protecting group itself serves as a useful, u.v.-sensitive chromophore. Under suitable circumstances, coupling of this derivative with other monomeric species may then be conducted, as well as a variety of chemical transformations that may be required, the sulfate function being liberated at the most appropriate stage in the sequence.

Phenyl chlorosulfate may also be of value in nucleotide chemistry, as nucleoside sulfates are stable, isosteric analogs. As removal of the phenyl group by hydrogenolysis may prove troublesome with purines, mild acid-hydrolysis could be preferable, or a nitrophenyl analog of the sulfating agent might merit investigation.

EXPERIMENTAL

General methods. — Solutions were usually evaporated below 40° under diminished pressure. Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Elemental analyses were performed by Guelph Chemical Laboratories Ltd., Guelph, Ont. Optical rotations were determined at room temperature, for solutions in 1-dm tubes, with a Perkin–Elmer polarimeter (model 141). I.r. spectra were recorded with a Perkin–Elmer 298 infrared spectrophotometer. Sheets of Eastman chromagram silica gel with fluorescent indicator were used for t.l.c. Mass spectra were recorded at the Biomedical Mass Spectrometry Unit, McGill

*By contrast, with ether as the solvent, ~75% of a chlorophenol was obtained.

University. Proton magnetic resonance spectra were recorded with a Varian T-60 or XL-200 spectrometer. Chemical shifts (δ) are reported with reference to tetramethylsilane.

Solvents. — Pyridine and benzene were stored over Linde 4A molecular sieves. Oxolane was refluxed with benzophenone and sodium under nitrogen, and distilled onto Linde 4A molecular sieves. *N,N*-Dimethylformamide and triethylamine were distilled from barium oxide, and the former was stored over Linde 4A molecular sieves.

Phenyl chlorosulfate. — To a solution of phenol (32 g, 0.34 mol) in methanol (50 mL) was added sodium hydroxide (13.3 g, 0.33 mol) dissolved in 85% methanol (100 mL), and the solution was stirred under a nitrogen atmosphere for 5 min, and evaporated. The residue was crushed, and dried at 55° under diminished pressure, affording sodium phenoxide as a light-brown powder, m.p. >200°. A slurry of the phenoxide (34.8 g, 0.30 mol) in benzene (150 mL) was added during 30 min to a stirred solution of sulfuryl chloride (24 mL, 0.30 mol) in benzene (100 mL) maintained at 0° under a nitrogen atmosphere. Insoluble material was filtered off, and the filtrate was washed with water, dried (anhydrous sodium sulfate), and evaporated, affording a brown oil (22 g). Distillation of the oil (40 g) through a Vigreux column gave fraction *i*, b.p. <60°/50 μm Hg, 9.0 g, of ~80% purity (^{1}H-n.m.r. evidence), and fraction *ii*, b.p. 61–65°/50 μm Hg, 19.0 g, of >90% purity (^{1}H-n.m.r. evidence); d 1.39; ν_{max} 1410 and 1195 cm^{-1} (SO_2); δ 7.3.

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose 3-(phenyl sulfate) (**3**). — A solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**; 1.30 g, 5.0 mmol) in oxolane (25 mL) was added during 30 min to a stirred suspension of sodium hydride (0.31 g, 6.5 mmol) in the same solvent (50 mL) under a nitrogen atmosphere. After 1 h, phenyl chlorosulfate (0.7 mL, 5.0 mmol) was introduced, and then stirring was continued for 20 h, the suspension was filtered, and the filtrate was evaporated. Chloroform was added, and the solution was washed successively with water, M hydrochloric acid, water, saturated sodium hydrogencarbonate, and water, dried (anhydrous sodium sulfate), and evaporated, to give a pale-yellow oil (1.55 g, 75%): R_F 0.72 (2:1 ethyl acetate–petroleum ether); ν_{max}^{neat} 1413 and 1210 cm^{-1} (SO_2); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.35 (m, 5 H, C_6H_5), 5.90 (d, 1 H, H-1), 5.20 (d, 1 H, H-3), 4.85 (d, 1 H, H-2), 4.4–3.9 (m, 4 H, H-4,5,6,6'), 1.45 (s, 3 H, CCH_3), 1.38 (s, 3 H, CCH_3), and 1.25 (s, 6 H, CMe_2); $J_{1,2}$ 3.5, $J_{3,4}$ <3 Hz.

Anal. Calc. for $C_{18}H_{24}O_9S$: mol. wt., 416. Found: *m/z* 401 (100%) (M − CH_3): *m/z* 403 (6.9%) (M + 2 ^{34}S − CH_3).

1,2:3,5-Di-O-methylene-α-D-glucofuranose 6-(phenyl sulfate) (**6**). — 1,2:3,5-Di-*O*-methylene-α-D-glucofuranose (**5**; 1.02 g, 5.0 mmol) was treated with sodium hydride and phenyl chlorosulfate as for the *O*-isopropylidene derivative, affording compound **6** as an oil (1.38 g, 77%); R_F 0.73 (ethyl acetate), 0.70 (ether); ν_{max}^{neat} 1410 and 1208 cm^{-1} (SO_2); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.4 (m, 5 H, C_6H_5), 6.1 (d, 1 H, H-1), and 5.2–4.0 (H-2–6', 2 CH_2); $J_{1,2}$ 3.5 Hz.

Anal. Calc. for $C_{14}H_{16}O_9S$: mol. wt., 360. Found: *m/z* 360 (M).

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose 3-(sodium sulfate) (**4**). — To a solution of **3** (0.63 g, 1.5 mmol) in 19:2 ethanol–water were added potassium carbonate (0.62 g, 4.5 mmol) and platinum oxide (80 mg), and the suspension was shaken for 20 h in a Parr hydrogenation apparatus under a hydrogen atmosphere at 37 lb.in.$^{-2}$. The suspension was filtered, the filtrate was evaporated, the residue was extracted with chloroform, and the extract was evaporated. Water was introduced, giving a milky suspension that was washed with ether, and the aqueous layer was evaporated, to afford a white solid (0.41 g); R_F 0.80 (ethanol), 0.60 (acetone). The product (0.38 g), in water (1 mL) was applied to a column of Dowex-1 X-8 (Cl^-) ion-exchange resin (50 mesh; 10 g), eluted with M ammonium hydrogencarbonate (total vol., 400 mL), and recovered by evaporation. Acetone was introduced, insoluble material was filtered off, and the filtrate was evaporated, giving the ammonium salt as a hygroscopic powder (0.22 g). As the latter proved difficult to handle, it was converted into the sodium salt (**4**) with Dowex 50-W X-8 (Na^+) ion-exchange resin, and the salt was purified as before by extraction into acetone. Evaporation of the acetone extract afforded white, lustrous crystals of **4**, m.p. >200° (dec.), $[\alpha]_D$ ~0° (*c* 2, water); ^{1}H-n.m.r. data (D_2O): δ 5.95 (d, 1 H, H-1), 4.85 (d, 1 H, H-2), 4.75 (d, 1 H, H-3), 4.4 (m, 2 H, H-4,5), and 4.0 (m, 2 H, H-6,6').

Anal. Calc. for $C_{12}H_{19}NaO_9S \cdot H_2O$: C, 37.9; H, 5.6; S, 8.4. Found: C, 38.2; H, 5.5; S, 8.3.

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