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# An improved preparation of cyclohexylammonium allyl and D-glycer-1′-yl 6-deoxy-6-*C*-sulfonato-α-D-glucopyranosides

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**Abstract**

Potassium 6-deoxy-6-*C*-sulfonato-D-glucopyranose (sulfoquinovose) was refluxed with allyl alcohol and Dowex-50 ($H^+$) to give allyl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside (**1**), crystallised as the cyclohexylammonium salt. Oxidation of this by permanganate gave DL-glycer-1′-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside, crystallised as the brucinium salt. Conversion of the latter to the cyclohexylammonium salt allowed crystallisation of glycer-1′-yl 6-deoxy-6-*C*-sulfonato-α-D-glucopyranoside (3-α-D-sulfoquinovosyl-*sn*-glycerol) (**2**) as almost entirely the D-glyceryl diastereoisomer. 

*Keywords:* 6-Deoxy-6-*C*-sulfo-D-glucopyranose; Sulfoquinovose; Allyl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside; Allyl α-sulfoquinovoside; D-Glycer-1′-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside; D-Glyceryl α-sulfoquinovoside; 3-α-D-sulfoquinovosyl-*sn*-glycerol

## 1. Introduction

Continuing work on the metabolism of the plant sulfolipid, sulfoquinovosyldiacylglycerol [1], required D-glycer-1′-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside (D-glyceryl α-sulfoquinovoside, 3-α-D-sulfoquinovosyl-*sn*-glycerol) which Miyano and Benson [2] obtained in 1962 by permanganate oxidation of allyl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside (allyl α-sulfoquinovoside) (**1**). The latter was prepared by refluxing sulfoquinovose, as the anhydrous sulfonic acid, in allyl alcohol. In our hands this preparation [2] of the anhydrous acid was accompanied by extensive degradation and a less drastic procedure was sought. This is described below, as is a simplified procedure for the oxidation by potassium permanganate of allyl α-sulfoquinovoside to DL-glycer-1′-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside followed by the crystallisation of cyclohexylammonium D-glycer-1′-yl 6-deoxy-6-*C*-sulfonato-α-D-glucopyranoside (D-glyceryl α-sulfoquinovoside) (**2**).

## 2. Results and discussion

Sulfoquinovose, as the potassium salt, was refluxed with Dowex-50($H^+$) in allyl alcohol to

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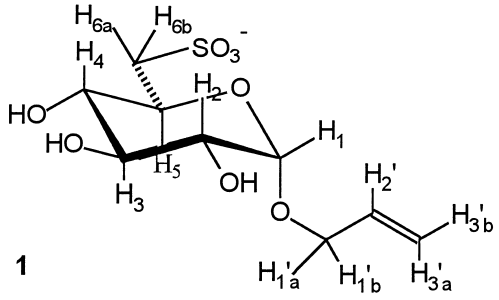


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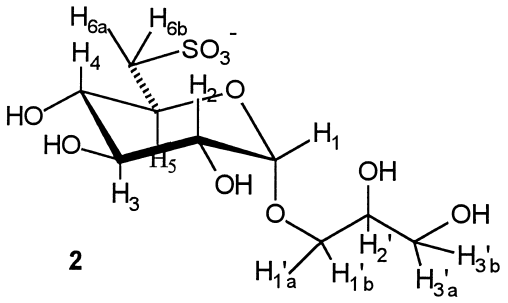


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give allyl 6-deoxy-6-*C*-sulfonato-α-D-glucopyranoside (**1**), isolated as the cyclohexylammonium salt in good yield (63% compared with 55% in [2]). The procedure was milder than that in [2] and the work-up of the mother liquors from the first crystallisation of the α-sulfoquinovoside was simpler. $^{1}H$ and $^{13}C$ NMR spectroscopy (Tables 1 and 2) confirmed the purity of the glycoside and also the $^{13}C$ assignments made by Johns et al. [3] on comparative grounds.

$^{13}C$ NMR spectroscopy of the products of the oxidation of allyl α-sulfoquinovoside by permanganate showed the absence of allyl signals. In the anomeric region two signals (δ 98 ppm, approximately equal intensity) predominated, consistent with the formation of the D- and L-glyceryl sulfoquinovosides. Other signals (δ ~102 ppm) together with a minor signal at δ 177 ppm were possibly derived from an over-oxidation product such as a sulfoquinovoside of glyceric acid. The brucinium salt of DL-glycer-1′-yl α-sulfoquinovoside was crystallised in good yield and by a procedure which avoided the tedious chromatography and repeated evaporations in ref. [2]. Conversion to the cyclohexylammonium salt allowed crystallisation [2] of cyclohexylammonium D-glycer-1′-yl 6-deoxy-6-*C*-sulfonato-α-D-glucopyranoside (**2**) containing about 10% of the L-glyceryl sulfoquinovoside. The amount of the latter was not decreased by recrystallisation. The assignment of the D-glycer-1′-yl structure in ref. [2]. depended solely on the observation that the melting point was not depressed on mixing with the glycoside of natural origin which had been characterised by degradation to L-glyceric acid [4] and subsequently by crystallography [5]. Small amounts of the L-glyceryl glycoside would probably not have been detected in ref. [2]. The configuration of the glyceryl residue in the

Table 1
$^{1}H$ NMR chemical shifts (ppm) and coupling constant(s) (Hz) for cyclohexylammonium allyl and D-glycer-1′-yl 6-deoxy-6-sulfonato-α-D-glucopyranosides. The resonances of cyclohexylammonium were as expected and are not shown

| | Chemical shifts and coupling constants | |
|---|---|---|
| | Allyl glycoside | Glyceryl glycoside |
| H-1 | 4.77 d | 4.72 d |
| | J 3.7 | J 3.8 |
| H-2 | 3.43 dd | 3.42 |
| | J 3.8, 9.8 | Obscured |
| H-3 | 3.56 t | 3.57 t |
| | J~9.5 | J~9.5 |
| H-4 | 3.10 t | 3.19 t |
| | J~9.5 | J~9.4 |
| H-5 | 3.92 | 3.89 t (br) |
| | Obscured | J 9.8 |
| H-6a | 3.22 dd | 3.22 d (br) |
| | J 14.7, 1.5 | J 14.6 |
| H-6b | 2.90 dd | 2.90 dd |
| | J 14.7, 9.9 | J 14.6, 9.8 |
| H-1′a | 4.19 m* | 3.27 dd |
| | | J 10, 7 |
| H-1′b | 3.92 m* | 3.78 dd |
| | | J 10, 4 |
| H-2′ | 5.83 m* | 3.82 m |
| H-3′a | 5.24 m* (cis) | 3.43 (Obscured) |
| H-3′b | 5.12 m* (trans) | 3.53 dd |
| | | J 12, 4 |

* All multiplets typical for allyl.

Table 2
$^{13}C$ NMR chemical shifts (p.p.m.) for cyclohexylammonium allyl and D-glycer-1′-yl 6-deoxy-6-sulfonato-α-D-glucopyranosides. The resonances for cyclohexylammonium were as expected and are not shown

| | Chemical shifts | |
|---|---|---|
| | Allyl glycoside | Glyceryl glycoside |
| C-1 | 96.6 | 98.15 |
| C-2 | 71.1 | 71.3 |
| C-3 | 73.0 | 72.8 |
| C-4 | 72.5 | 72.35 |
| C-5 | 67.9* | 68.0 |
| C-6 | 52.1 | 51.9 |
| C-1′ | 68.0* | 68.8 |
| C-2′ | 133.4 | 70.7 |
| C-3′ | 118.4 | 62.5 |

* These assignments may be reversed.

present preparation of glyceryl α-sulfoquinovoside has not been established but the melting point is that of the D isomer [2]. In the analogous pairs of D- and L-glycer-1′-yl α- and β-D-galactopyranosides the melting points differ by nearly 30 °C while $[\alpha]_D$, on the other hand, is not a useful distinguishing character [6]. $^1H$ and $^{13}C$ NMR data are given in Tables 1 and 2: the assignments of the $^{13}C$ resonances agree with those made previously [3]. In the latter work the stereochemistry of the glyceryl residue was not specified and the signals from the pair of anomeric carbon atoms would not have been resolved under the conditions then available [3].

$^{13}C$ NMR spectroscopy showed that the major component of the residues from the crystallisation of cyclohexylammonium D-glycer-1′-yl α-sulfoquinovoside was the corresponding L-glycer-1′-yl derivative but attempts to crystallise this were unsuccessful.

## 3. Experimental

*General methods*.—Rotary evaporation was at a bath temperature of 45 °C. Compounds were dried in vacuo over $P_2O_5$ at room temperature. Optical rotations were measured at 546 nm and at 20 °C in a Thorn-NPL polarimeter (model 243) with a 10 mm light path. TLC was carried out as before [7] and glycosides were detected using alkaline $KMnO_4$.

*NMR*.—Spectra were recorded in $D_2O$ at 20 °C on a Bruker AMX 360 spectrometer operating at 360 MHz for $^1H$ and 90 MHz for $^{13}C$. Chemical shifts are given relative to sodium 3-trimethylsilylpropanesulfonate used as an external standard and assigned δ 0 for $^1H$ and δ −2.6 for $^{13}C$. $^1H$ Chemical shift assignments were confirmed by COSY spectra and $^{13}C$ assignments were established from $^{13}C$–$^1H$ correlation 2D spectra. Standard Bruker software was used throughout.

*Cyclohexylammonium allyl 6-deoxy-6-C-sulfonato-α-D-glucopyranoside*.—Potassium 6-deoxy-6-*C*-sulfonato-D-glucopyranose (sulfoquinovose: 2.1 g, 7 mmol) [7] and 4 g of Dowex-50×4($H^+$) (exhaustively washed in allyl alcohol) were refluxed with 200 mL of allyl alcohol (refluxed over and distilled from $CaSO_4$) for 4 h. The allyl alcohol solution of the sulfoquinovosides was removed from the resin and unreacted sulfoquinovose and retained. To the residual solids were added 100 mL of allyl alcohol and 2 g of Dowex-50($H^+$) and the mixture refluxed for 4 h by which time very little sulfoquinovose remained. The allyl alcohol was removed, combined with the previous reaction mixture, and with allyl alcohol washings of the resin, then filtered. The filtrate was concd to ∼20 mL and diluted to 200 mL with water before neutralising with 0.5 M cyclohexylamine: close to the theoretical amount was required. This soln was concd to a thin syrup which was dild with 10 mL of water and filtered. The filtrate was again concd to a syrup which was dissolved in EtOH (20 mL) and concd: repeating this procedure gave the solid sulfoquinovosides (2.8 g) which contained, as judged by $^1H$ NMR, ∼65 and 25%, respectively, of α and β pyranosides and ∼10% of what was perhaps a furanoside. Three compounds reacting with $KMnO_4$, $R_f$ 0.56, 0.65 and 0.77 in order of their decreasing abundance, were detected by TLC (system 3 in ref. [7]).

This product was dissolved in hot EtOH (5 mL) and EtOAc (∼9 mL) was slowly added to give a faint turbidity in the boiling soln. Crystallisation occurred on slow cooling to room temperature and after keeping overnight at 5 °C the white crystals were filtered off, washed with EtOH–EtOAc (10:6) and dried. Yield 1.2 g (3.1 mmol, 44%). NMR spectroscopy showed only the α anomer.

The filtrate and washings from the crystals were concd to dryness to give a solid (1.4 g) which contained ($^1H$ NMR spectroscopy) approximately equal amounts of the α- and β-sulfoquinovosides. It was dissolved in allyl alcohol (20 mL) and refluxed with 2 g of Dowex-50($H^+$) for 8 h. Work-up as above gave a further 0.36 g of crystals. The filtrate and washings from this second crop gave 1.0 g of a glassy solid on drying: treatment of this as above with 20 ml of allyl alcohol and 1 g of Dowex-50($H^+$) gave a further 0.15 g of product. These three crops of crystals showed only a single component, $R_f$ 0.56, on TLC and they were therefore combined to give a total yield of 1.7 g (4.4 mmol, 63%).

TLC of the dark brown filtrate from the last crystallisation detected approximately equal amounts of the three components of the original reaction mixture, a finding confirmed by $^1H$ NMR spectroscopy.

Recrystallisation from EtOH (∼4 mL/g) of the combined products gave white crystals (1.2 g) of the title compound (**1**). m.p. 162–164 °C d, lit. 151.5–152 °C [2]; $[\alpha]_{546}^{20}$ +102° (*c* 1, $H_2O$), lit. $[M]_D^{25}$ +32967° ($[\alpha]_D^{25}$ +86.0°) [2]; $^1H$ and $^{13}C$ NMR data are given in Tables 1 and 2; Anal.

Calcd for $C_{15}H_{29}NO_8S$: C, 47.0; H, 7.62; N, 3.65. Found: C, 47.2; H, 7.80; N, 3.76.

*Brucinium* DL-*glycer-1′-yl 6-deoxy-6*-C-*sulfonato*-α-D-*glucopyranoside*.—Cyclohexylammonium allyl 6-deoxy-6-*C*-sulfonato-D-glucopyranoside (0.96 g, 2.5 mmol) in 5 mL of water was converted to the acid by passage through a column of Dowex-50×4($H^+$) and the soln was neutralised to pH 7 with NaOH before taking to dryness. To the residue in 5 mL of water was added 0.25 mL of 1 M NaOH and the soln stirred in ice: 435 mg (2.75 mmol) of $KMnO_4$ in 20 mL of cold water was then added dropwise over 1 h. The mixture was stirred in ice for 2 h and left overnight at room temperature before filtering and washing the residue thoroughly with hot water. TLC of the colorless filtrate showed complete utilisation of the allyl sulfoquinovoside and the appearance of a compound with $R_f$ 0.18 together with two very minor components, $R_f$ 0.05 and 0.02. The soln was concd to ~5 mL, passed through a column of Dowex-50($H^+$) and the brucinium salt prepared as described for sulfoquinovose [7], giving 1.6 g of a white solid (2.2 mmol, 90%).

The salt was crystallised twice from 90% ethanol (3 then 6 mL/g) to give 0.82 g of the title compound as white, slightly hygroscopic crystals. TLC (50 nmol in systems 2 and 3 [7]) gave single spots, $R_f$ 0.18 and 0.66, respectively (brucinium moved separately and was also detected by $KMnO_4$). m.p. 173–175 °C d; $[\alpha]_{546}^{20}$ +22° (*c* 1.5 $H_2O$); $^1H$ NMR ($D_2O$): $\delta$ 4.7 ppm (overlapping pair of doublets, $J$~4 Hz H-1$\beta$ of the D- and L-glyceryl α-sulfoquinovosides) Anal. Calcd for $C_{32}H_{44}N_2O_{14}S.H_2O$: C, 52.6; H, 6.34; N, 3.83. Found C, 52.5; H 6.55; N, 3.94.

*Cyclohexylammonium* D-*glycer-1′-yl 6-deoxy-6*-C-*sulfonato*-α-D-*glucopyranoside*.—Brucinium DL-glycer-1′-yl 6-deoxy-6-*C*-sulfonato-α-D-glucopyranoside (0.55 g, 0.75 mmol) in 4 mL of water was converted to the free acid and then to the cyclohexylammonium salt which was obtained as a glass on concn. This was dissolved in EtOH and concd: repeating this gave the solid cyclohexylammonium salt (0.33 g).

This was dissolved in ~1 mL of EtOH at ~55 °C and toluene (~0.7 mL) added slowly to the warm solution until a faint turbidity developed. Slow cooling to room temperature gave two liquid layers which solidified at 5 °C. The mixture was warmed to ~55 °C and EtOH (~3 mL) added dropwise until complete solution was achieved: fine white needles rapidly separated on cooling. After standing at −20 °C, these were filtered off, washed with cold EtOH and dried to give 87 mg (0.21 mmol, 28%) of the title compound (**2**). m.p. 188–189 °C d, lit. 191–193 °C [2]; $[\alpha]_{546}^{20}$ +88.7° (*c* 1, $H_2O$), lit. $[\alpha]_D^{25}$ +74.5° [8]; $^1H$ and $^{13}C$ NMR data are given in Tables 1 and 2; Anal. Calcd for $C_{15}H_{31}NO_{10}S$: C, 43.2; H, 7.48; N, 3.36. Found: C, 43.1; H, 7.10; N, 3.41.

A sample (75 mg) was recrystallised from EtOH (4 mL) in 15% yield. This did not decrease the small amount of L-glyceryl sulfoquinovoside which was present.

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