ELSEVIER

Carbohydrate Research 302 (1997) 113–117

CARBOHYDRATE RESEARCH

Note

Sulfoquinovose and its aldonic acid: their preparation and oxidation to 2-sulfoacetaldehyde by periodate

Alexander B. Roy [a,*], Michael J.E. Hewlins [b]

[a] *School of Molecular and Medical Biosciences, University of Wales Cardiff, Cardiff CF1 3US, UK*
[b] *Department of Chemistry, University of Wales Cardiff, Cardiff CF1 3US, UK*

Received 22 July 1996; accepted 14 March 1997

Abstract

6-Deoxy-6-*C*-sulfo-D-glucopyranose (sulfoquinovose) has been prepared and characterised as its brucinium and potassium salts. The bis-cyclohexylammonium salt of its aldonic acid, 6-deoxy-6-*C*-sulfo-D-gluconic acid, has also been obtained. Their oxidation by sodium periodate gives 2-sulfoacetaldehyde. © 1997 Elsevier Science Ltd.

Keywords: 6-Deoxy-6-*C*-sulfo-D-glucopyranose; Sulfoquinovose; 6-Deoxy-6-*C*-sulfo-D-gluconic acid; Periodate oxidation; 2-Sulfoacetaldehyde

1. Introduction

Sulfoquinovose (6-deoxy-6-*C*-sulfo-D-glucopyranose) is the defining constituent of the plant sulfolipid (sulfoquinovosyldiacylglycerol) [1] that is universally associated with oxygenic photosynthesis [2] and an important component of the biological sulfur cycle [3].

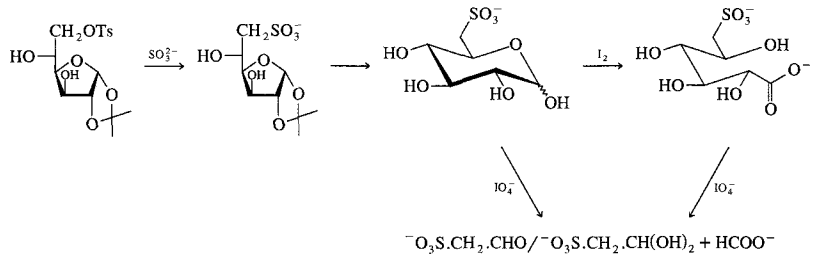
In connection with studies of sulfolipid metabolism substantial quantities of sulfoquinovose were needed. Although crystalline sulfoquinovosides have been described [4–6] the parent sugar is neither so well known nor characterised [7,8]. Information about the oxidation by periodate of sulfoquinovose and its aldonic acid was also required. We now report the preparation of sulfoquinovose (based on work by Miyano and Benson [4]), characterisation of its brucinium and potassium salts, conversion into the aldonic acid and oxidation by periodate of both the aldose and the aldonic acid to sulfoacetaldehyde (Scheme 1).

2. Results and discussion

The sulfoquinovose was first obtained as an equimolecular mixture of its barium salt with barium *p*-toluenesulfonate: Miyano and Benson [4] removed the latter by extraction with hot 95% ethanol but did not purify the sugar. Separation of the potassium salts, as described below, was simpler and the sulfoquinovose was purified through its brucinium salt which was shown by ^{1}H NMR spectroscopy to crystallise predominantly as the α anomer [H-1α, δ 5.19 (J 3.2 Hz), H-1β, δ 4.65 (J 8.1 Hz)]. The brucinium salt was converted into the potassium salt and precipitated from aqueous solution by ethanol. Its NMR resonances, given in Tables 1 and 2, are consistent

* Corresponding author.

PII S0008-6215(97)00112-2



Scheme 1.

with the pyranose structure, and the ^{13}C NMR data, assigned unequivocally from the ^{13}C–^{1}H correlation 2D-NMR spectrum, confirm the assignments made by Johns et al. [9] from the study of model compounds. The α/β ratio was 32:68. Crystallisation [7] gave a preparation containing, from its NMR spectrum, about 85% of the anomer some 15 min after dissolving in D_2O and about 33% at equilibrium.

The potassium salt, suitable for preliminary biological studies, was easily obtained by an alternative procedure avoiding the preparation of the brucinium salt. TLC detected only sulfoquinovose, and the ^{1}H NMR spectrum showed only the resonances seen with the purified sample.

Oxidation of sulfoquinovose by hypoiodite gave 6-deoxy-6-*C*-sulfo-D-gluconic acid, crystallised as the bis-cyclohexylammonium salt. NMR data are given in Tables 1 and 2.

2-Sulfoacetaldehyde (2-oxoethanesulfonic acid), the expected product of the oxidation by periodate of sulfoquinovose or its aldonic acid, was prepared as the bisulfite adduct [10]. The ^{1}H and ^{13}C NMR results (Table 3) are as expected. They confirm the facile exchange in D_2O of the protons at C-2, as previously found by mass spectrometry [10], and they are in agreement with values reported by White [10] except in the following points. We find H-1 as a double doublet at δ 4.82 α ppm while White [10], at 270 MHz, reported a doublet at δ 4.28. Presumably the 1.5-Hz coupling was not resolved at the lower field strength and we attribute the apparent difference in chemical shift to a typographical error. Further, White's ^{13}C data (unspecified conditions) showed the non-decoupled C-2 signal as a 'quartet' while we observe an expected triplet. A possibility that White's spectrum showed a double doublet, due to different couplings to the two diastereotopic protons, seems unlikely if a lower field strength was used than in our case.

Table 3 also gives data for 2-sulfoacetaldehyde, which show that the hydrated form is the dominant species in solution.

As expected from the behaviour of 6-deoxyhexoses, oxidation of sulfoquinovose or its aldonic acid with sodium metaperiodate gave 2-sulfoacetaldehyde from carbon atoms 5 and 6. The ^{13}C NMR spectrum of the reaction mixture from sulfoquinovose in water showed the resonances of both carbon atoms of sul-

Table 1
^{1}H NMR data (D_2O) for the potassium salts of sulfoquinovose and its aldonic acid

Atom	Chemical shifts (δ) and coupling constants (Hz)		
	Sulfonatoquinovose		Aldonate
	α Anomer	β Anomer	
H-1	5.2 d	4.6 d	
	J 3.7	*J* 8	
H-2	3.54 dd	3.26 dd	4.09 d
	J 3.7, 9.7	*J* 8, 9.4	*J* 3.5
H-3	3.72 t	3.49 t	4.02 t
	J 9.7	*J* 9.3	*J* 3.5
H-4	3.27 t	3.27 t	3.69 dd
	J ~9.8	*J* ~9.4	*J* 6.7, 3.5
H-5	4.21 td	3.79 td	4.15 dd
	J 9.8, 1.5	*J* 9.5, 1.5	*J* ~7, ~9
H-6a	3.4 dd	3.40 dd	3.31 d
	J 15, 1.5	*J* 15, 1.5	*J* 14.5
H-6b	3.06 dd	3.07 dd	3.01 dd
	J 9.8, 15	*J* 9.6, 15	*J* 14.5, 9.7

Table 2
^{13}C NMR data (water) for the potassium salts of sulfoquinovose and its aldonic acid

Atom	Chemical shifts (δ)		
	Sulfonatoquinovose		Aldonate
	α Anomer	β Anomer	
C-1	92.95	96.9	178.5
C-2	72.4	75.0	73.9
C-3	[73.65	76.5	70.8
C-4	[73.6	73.3	74.2
C-5	68.7	73.1	67.8
C-6	53.3	53.2	53.5

Table 3
NMR data for the potassium salts of the bisulfite adduct of sulfoacetaldehyde and of the corresponding aldehyde. The proton spectra were measured in D_2O and the carbon spectra in water or D_2O as specified

Atom	Chemical shifts (δ) and coupling constants (Hz)		
	Adduct		Aldehyde
	Present work	Data of White [10]	
H-1	4.82 dd *J* 10.2, 1.5	4.28 d *J* 10.2	5.3, 9.5 [a]
H-2a	3.48 dd *J* 14.5, 1.5	3.51 d *J* 14.3	[b]
H-2b	3.20 dd *J* 10.2, 14.5	3.24 dd *J* 10.2, 14.3	[b]
C-1	80.4 [c]	85 [c]	87.3 [c]
C-2	52.4 [d]	54.8 [e]	57.4 [d,f]

[a] The first resonance is that of the diol: the second, ten-fold weaker, that of the aldehyde.
[b] The exchange of these protons with D_2O is too rapid to allow their detection.
[c] A doublet in the non-decoupled spectrum.
[d] A triplet in the non-decoupled spectrum.
[e] A quartet in the non-decoupled spectrum.
[f] Not seen when the adduct is decomposed in D_2O.

foacetaldehyde (Table 3) but after oxidation in D_2O only that of C-1, at 87 ppm, was detected because of the exchange of the C-2 protons. Formate, with a resonance at 170 ppm, was the only other product detected in either medium. 6-Deoxy-6-*C*-sulfo-D-gluconic acid also gave formate and sulfoacetaldehyde on oxidation: no resonance arising from CO_2 or HCO_3^- was detected because any signal from these would be inherently weak and CO_2 would rapidly be lost at the final pH of the reaction mixture (ca. 4.2).

3. Experimental

General methods.—Rotary evaporation was carried out at a bath temperature of 50 °C. Compounds were exhaustively dried over P_2O_5 in vacuo at room temperature. Optical rotations were measured at 546 nm and 20 °C in a Thorn-NPL polarimeter (model 243) with a 10-mm light path.

NMR.—Spectra were recorded at 20 °C on a Bruker AMX360 spectrometer operating at 360 MHz for ^1H and 90 MHz for ^{13}C. The solvents were D_2O or D_2O–H_2O mixtures, as noted elsewhere. Chemical shifts are given relative to sodium 4,4-dimethyl-4-silapentanesulfonate 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.

TLC.—Glass-backed plates of Silica Gel 60 (E. Merck, Poole, Dorset) were used in satd tanks: for solvent system 3 the plates were impregnated with phosphate [11] prior to use. The solvent systems were: 1, 10:1 butan-2-one–water; 2, 20:10:1 EtOH–water–HOAc; 3, 2:2:1 propan-2-ol–acetone–0.1 M formic acid.

A small sample of sulfoquinovose, previously prepared by the addition of bisulfite to methyl 6-deoxy-α-D-*xylo*-hex-5-enopyranoside [5], was available as a standard.

Crude barium salt of sulfoquinovose.—1,2-*O*-Isopropylidene-α-D-glucofuranose (26 g, 118 mmol) was converted into its 6-*O*-tosyl derivative [12] which was, without further purification, dissolved in EtOH (450 mL). Na_2SO_3 (27 g, 215 mmol) in water (450 mL) was added and the suspension refluxed for 24 h. TLC (system 1) showed complete utilisation of the tosyl derivative (R_f 0.39) and the appearance of a compound, $R_f \sim 0.1$, presumably 6-deoxy-1,2-*O*-isopropylidene-6-*C*-sulfo-α-D-glucofuranose (isopropylidenesulfoquinovose). Sufficient water was added to dissolve the excess of Na_2SO_3 and the soln evaporated to ~300 mL. Crystals of 3,6-anhydro-1,2-*O*-isopropylidene-5-*O*-tosyl-D-glucofuranose, from the unpurified 6-tosyl intermediate, separated and were discarded.

The filtrate was loaded on a column (5.5 × 43 cm) of Dowex 50-X8(H^+) (200–400 mesh) and eluted with water: the acid fraction (~600 mL) of the eluate was collected and concd to ~200 mL. TLC showed the main component to be sulfoquinovose but a small amount of its isopropylidene derivative remained. The concentrate was kept at 80 °C for 45 min to completely hydrolyse the acetal (cf. hydrolysis of isopropylideneglucose 6-sulfate [13]), dild with water (250 mL), and again concd to ~100 mL to remove most of the remaining SO_2. TLC (system 3) showed the resulting soln to contain sulfoquinovose (R_f 0.1) and small amounts of at least three reducing sugars.

The soln was dild to ~200 mL and its pH taken to 7.0 with $Ba(OH)_2$. After standing overnight, the precipitated inorganic salts of barium were removed by filtration and thoroughly washed with cold water. The filtrate and washings were concd to a paste containing the barium salts of sulfoquinovose and

p-toluenesulfonic acid, which was exhaustively dried over P_2O_5 in vacuo. Yield of mixture, 51 g.

Brucinium 6-deoxy-6-C-sulfonato-D-glucopyranose.—The mixed barium salts (17 g) were extracted with EtOH as previously described [4] to leave 5.8 g of the barium salt of sulfoquinovose. This was converted into the acid by passage through a column of Dowex 50-X8(H^+) as above and the appropriate fraction of the eluate taken to pH 6.7 by addition of a 20% soln of brucine in EtOH (~40 mL). After removing the EtOH by concentrating to ~75 mL, the aq soln was extracted three times with 25-mL portions of $CHCl_3$ and twice with 20-mL portions of Et_2O. Evaporation of the aq layer to dryness gave the crude brucinium salt (10.7 g).

The latter salt was dissolved in hot water (30 mL), the soln cooled to 40 °C, and Me_2CO, at the same temperature, added slowly until crystallisation began: 75 mL of Me_2CO were required. After cooling slowly to room temperature the mixture was kept at 5 °C until crystallisation was complete. The crystals were separated by filtration and washed with aq Me_2CO (proportions as above) and then Me_2CO. Yield, 8.7 g. The filtrate and washings were taken to dryness, the residue was dissolved in water (6 mL), and the soln treated as above to give a further 0.78 g of crystals.

TLC (system 3) detected only sulfoquinovose in both crops of crystals which were combined and recrystallised twice, with one treatment with activated carbon, by dissolving in water (~4 mL/g) and adding Me_2CO to initiate crystallisation (~3 mL/mL). Yield, 8.3 g of white crystals of the title compound; $[\alpha]_{546}^{20}$ −1.4 → −5.3° (*c* 3.5, H_2O: $t_{1/2}$ 160 min; TLC of 75 nmol (system 3) detected only sulfoquinovose. Anal. Calcd for $C_{29}H_{38}N_2O_{12}S \cdot 2H_2O$: C, 51.6; H, 6.27; N, 4.15. Found: C, 52.0; H, 6.75; N, 4.03.

Potassium 6-deoxy-6-C-sulfonato-D-glucopyranose.—The brucinium salt (1.9 g, 3 mmol in 20 mL of water) was converted into the acid by passage through a small column of Dowex 50-X4(H^+), the pH of the appropriate fraction of the eluate adjusted to 7.0 with KOH, and the soln taken to dryness. The potassium salt was dissolved in water (10 mL) and added dropwise through a filter to stirred EtOH (150 mL) chilled in ice. After standing overnight at 5 °C the suspension was filtered to give the potassium salt which was washed with cold EtOH and dried. Yield, 0.76 g (2.7 mmol, 89%) of the title compound as a white powder. TLC (system 3) of 100 nmol of the product, which showed only end-absorption in the UV ($\varepsilon_{210} < 10$), detected only sulfoquinovose. Anal. Calcd for $C_6H_{11}KO_8S \cdot H_2O$: C, 24.0; H, 4.36. Found: C, 24.0; H, 4.18.

To a soln of the above preparation (140 mg) in water (0.25 mL) at ~40 °C was added EtOH (120 μL) in 20-μL portions with constant shaking, until crystals began to appear. These increased on standing at room temperature for 48 h and, after chilling to 5 °C, were filtered off, washed with aq EtOH then EtOH, and dried. Yield, 90 mg; $[\alpha]_{546}^{20}$ +71.7 ± 1.0 → +49.7 ± 0.9° (*c* 1.5, H_2O and D_2O: $t_{1/2}$, 180 and 500 min, respectively), lit. $[\alpha]_D^{20}$ +59.5 → +38.2° [7].

Alternative preparation of the potassium salt of sulfoquinovose.—The barium salts of sulfoquinovose and *p*-toluenesulfonic acid (16 g) in water (50 mL) were converted into the acids by passage through Dowex 50(H^+) and the appropriate fraction of the eluate was brought to pH 7.0 with KOH. The volume was adjusted to 160 mL and the soln slowly added, with stirring, to EtOH (2.4 L) cooled in ice. After standing overnight at 5 °C the potassium sulfoquinovose was separated as described above. Yield, 6.4 g.

The product was reprecipitated twice from aq soln with EtOH (100 mL of soln, 1.5 L of EtOH), with one treatment with activated carbon, to give 4.9 g (78%) of the potassium salt as an off-white powder.

TLC (system 3) of 75 μg of the product detected only sulfoquinovose. UV spectroscopy showed a non-specific background absorption with a shoulder at 260 nm consistent with 0.8% contamination by a *p*-toluenesulfonyl derivative.

Bis-cyclohexylammonium 6-deoxy-6-C-sulfonato-D-gluconate.—To 0.5 mmol (150 mg) of sulfoquinovose (potassium salt) in 3 mL of water was added, while stirring, 0.1 mL of 0.4 M I_2 in MeOH followed by 0.2 mL of 0.6 M KOH in 50% MeOH. These additions were continued alternately until 2.5 mL of iodine soln (1 mmol) and 5 mL of KOH soln (3 mmol) had been added; then, after 30 min at room temperature, the reaction mixture was slowly added, with stirring, to 90 mL of EtOH chilled in ice. After some hours at 5 °C the precipitated dipotassium salt was collected, washed with EtOH then with Et_2O, and dried to a powder. The yield was quantitative.

The potassium salt was converted into the free acid by treatment with Dowex 50(H^+) and then into the bis-cyclohexylammonium salt which was obtained as a solid by evaporation. This solid (100 mg) was taken up in hot EtOH (5 mL), a small amount of insoluble material removed by hot filtration, and the filtrate concd to ~1 mL. Ethyl acetate (~1 mL) was

slowly added to the boiling ethanolic soln until a faint turbidity appeared. Crystals slowly formed on cooling: after 4 days at 5 °C these were filtered off, washed with cold EtOAc, and dried. Yield, 51 mg of the title compound; R_f 0.25 (solvent 2: cf. sulfoquinovose, R_f 0.64). Anal. Calcd for $C_{18}H_{38}N_2O_9S \cdot 0.5H_2O$: C, 46.2; H, 8.41; N, 5.99. Found: C, 46.3; H, 8.78; N, 5.99.

2 - Sulfoacetaldehyde.—This was prepared (56% yield) as the bisulfite adduct by the method of White [10]. Anal. Calcd for $C_2H_4Na_2O_7S_2 \cdot 2H_2O$: C, 8.39; H, 2.82. Found: C, 8.43; H, 3.11.

The aldehyde was obtained as required by treatment of the adduct with Ba^{2+} [14]. To a 0.1 M aq soln of the adduct was added an equal volume of 0.11 M $BaCl_2$ and the pH raised to 8 with 1.0 M NaOH: about 95% of the theoretical amount was required. The precipitate of $BaSO_3$ was removed by centrifugation and the soln used without further treatment. When the reaction was carried out in D_2O, the same procedure was used but 95% of the calculated amount of NaOD was added and the pD was not measured.

Periodate oxidations.—Titrimetric determinations, in 0.15 M $NaHCO_3$ [15], showed that the oxidation of 10 μmol of quinovose or of sulfoquinovose by 100 μmol of $NaIO_4$ consumed 39 and 38 μmol, respectively, of the latter (theory, 40 μmol). Subsequent oxidations were carried out in phosphate buffer [16], either in water or D_2O.

In water, the reaction mixture contained 50 μmol of deoxyhexose in 1.75 mL of 0.5 M phosphate buffer, pH 5.7 (86 mM Na_2HPO_4, 0.41 M $NaH_2PO_4 \cdot H_2O$), and 0.75 mL of 0.3 M $NaIO_4$ (225 μmol). After 1 h in the dark at room temperature, titration showed the oxidation to be complete and the reaction mixture was investigated by NMR spectroscopy.

In D_2O the general procedure was similar but the phosphate buffer, of the same composition, was made up in D_2O and exchanged several times with this solvent: the measured pD was 6.3. The $NaIO_4$ was also made up in D_2O. Again, titration showed the oxidation to be complete within 1 h, after which time the reaction mixture was taken for NMR studies.

Acknowledgements

A.B.R. is deeply indebted to Professor J.L. Harwood for his support of this work. Anne Dams is thanked for the elemental analyses.

References

[1] A.A. Benson, H. Daniel, and R. Wiser, *Proc. Natl. Acad. Sci. U.S.A.*, 45 (1959) 1582–1587.
[2] J.L. Harwood, in P.K. Stumpf and E.E. Conn. (Eds.), *Biochemistry Of Plants*, Vol. 4, Academic, New York, 1980, pp. 1–55.
[3] J.L. Harwood and R.G. Nicholls, *Biochem. Soc. Trans.*, 7 (1979) 440–447.
[4] M. Miyano and A.A. Benson, *J. Am. Chem. Soc.*, 84 (1962) 59–62.
[5] J. Lehmann and A.A. Benson, *J. Am. Chem. Soc.*, 86 (1964) 4469–4472.
[6] J. Lehmann and W. Weckerle, *Carbohydr. Res.*, 22 (1972) 23–35.
[7] H. Ohle and W. Mertens, *Ber.*, 68 (1935) 2176–2187.
[8] B. Helferich and W. Ost, *Hoppe-Seyler's Z. Physiol. Chem.*, 331 (1963) 114–117.
[9] S.R. Johns, D.R. Leslie, R.I. Willing, and D.G. Bishop, *Aust. J. Chem.*, 31 (1978) 65–72.
[10] R.H. White, *Biochemistry*, 27 (1988) 7458–7462.
[11] S.A. Hansen, *J. Chromatogr.*, 107 (1975) 224–226.
[12] J.R. Snyder and A.S. Serianni, *Carbohydr. Res.*, 163 (1987) 169–188.
[13] P.J. Archbald, M.D. Fenn, and A.B. Roy, *Carbohydr. Res.*, 93 (1981) 177–190.
[14] H. Kondo, H. Anada, K. Ohsawa, and K. Ishimoto, *J. Biochem. (Tokyo)*, 69 (1971) 621–623.
[15] J.R. Dyer, *Methods Biochem. Anal.*, 3 (1956) 111–152.
[16] J.K.N. Jones and R.J. Stoodley, *Methods Carbohydr. Chem.*, 2 (1963) 489–493.