SYNTHESIS OF DL-[2-^{14}C]OCTAN-2-SULPHATE and [^{35}S]-LABELLED D(+)- and L(-)-OCTAN-2-SULPHATE

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SUMMARY

DL-[2-^{14}C]Octan-2-sulphate was prepared by sulphating DL-[2-^{14}C]octan-2-ol with pyridine-SO_3 adduct. Synthesis of the DL-[2-^{14}C]alkanol started with carboxylation of hexyl magnesium bromide with $^{14}CO_2$. Subsequent methylation with diazomethane and reduction of the ester with lithium aluminium hydride yielded [1-^{14}C]heptan-1-ol. The latter was oxidized to [1-^{14}C]heptanal with Seloxcette. The [1-^{14}C]heptanal was reacted with methyl magnesium iodide to give DL-[2-^{14}C]octan-2-ol. D(+)- and L(-)-Octan-2-[^{35}S]sulphate were synthesized by sulphating the stereochemically pure alcohols with pyridine-$^{35}SO_3$ prepared from $^{35}SO_3$. These synthetic routes provided specifically radiolabelled secondary alkyl sulphates of high chemical and radiochemical purity. The identity and purity of the products was confirmed by mass spectrometry, infrared spectroscopy, t.l.c. and stereospecific enzymic hydrolysis.

Key words: 2-carbon-14, sulphur-35, octyl-2-sulphate, optical isomers, Seloxcette

INTRODUCTION

Secondary alkyl sulphates are employed as surfactants in commercial formulations that have a number of industrial applications (1). In order to carry out a thorough investigation of the metabolic fate of a selected secondary alkyl sulphate, octan-2-sulphate was prepared in the following radiolabelled forms: DL-[2-^{14}C], D(+)-[^{35}S] and L(-)-[^{35}S]. The use of both radiolabels enabled studies to be carried out on the metabolic fate of both the carbon skeleton and

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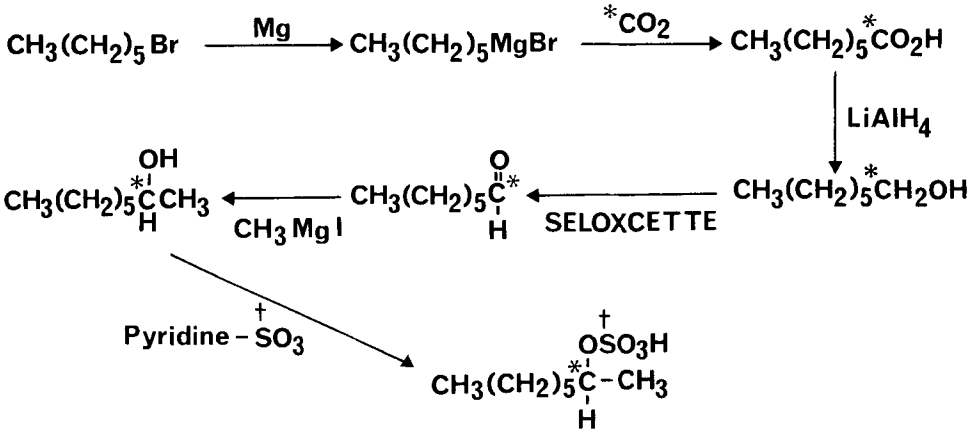
the sulphate moiety. The insertion of the ^{14}C-label at carbon 2 was carried out in order to minimize the loss of ^{14}C as $^{14}CO_2$ by ω,β-oxidation since oxidation of the carbon atom adjacent to the sulphate moiety does not generally occur in vivo (2). Stereospecific syntheses enabled the metabolic fate of stereoisomers to be investigated in order that any stereoselectivity of enzyme systems involved in the detoxication of secondary alkyl sulphates could be determined. The secondary alkyl sulphates were synthesized by sulphating the corresponding alcohols with pyridine-SO_3 adduct, a reagent known to sulphate secondary alkanols without causing positional isomerization or racemization (3). [2-^{14}C]Octan-2-ol was prepared by the route shown in the Figure. The preparation of [1-^{14}C]heptan-1-ol was based on a previously reported procedure for the synthesis of [1-^{14}C]alkanols (4). A method for preparing pyridine-$^{35}SO_3$ which was suitable for sulphating secondary alcohols was developed in which no pyridinium chloride was present in the final pyridine-$^{35}SO_3$ reagent. This contaminant has been suggested (5) to have a detrimental effect if present during the sulphation reaction. This method enabled the synthesis of stereochemically pure radiolabelled enantiomers.

Figure



* denotes ^{14}C label

† denotes ^{35}S label

MATERIALS

1-Bromohexane was purchased from B.D.H. Ltd., Poole, Dorset. U.K. Heptanal was obtained from Ralph N. Emanuel Ltd., Wembley, England. Heptan-1-ol, DL-octan-2-ol and D(+)- and L(-)-octan-2-ol were products of Koch-Light Laboratories Ltd., Colnbrook, Bucks, U.K. Seloxcette (CrO_3-graphite, 55.2: 44.8, by wt., 20 mesh) was obtained from Alfa Products, Beverly, Mass., U.S.A. $Ba^{14}CO_3$ (20mCi/mmol.) and $^{35}SO_3$ (3.76mCi/mmol.) were supplied by The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were purchased from standard commercial sources.

ANALYTICAL TECHNIQUES

Thin-layer chromatography

Chromatography was performed on silica-gel G. Octan-2-ol was chromatographed in ether:hexane (1:9, by vol.). Chromatograms of octan-2-sulphate were developed with chloroform:methanol:water (65:25:4, by vol.), propan-2-ol: 17M-ammonia (7:3, by vol.) and 2-methyl propanoic acid (isobutyric acid): 0.5M-ammonia (5:3, by vol.). Radioactivity on chromatograms was located by autoradiography and quantitated by liquid scintillation counting in a Packard 2425 Tri-Carb liquid-scintillation spectrometer.

Infra-red spectroscopy

Samples of octan-2-sulphate were examined as KBr discs in a Perkin-Elmer 137B Infracord spectrophotometer.

Radio-gas-liquid chromatography

A Pye 104 gas chromatograph was linked to a Panax radio-gas detector. The glass column (1.5m x 4mm i.d.) was packed with 10% (w/w) Carbowax 20M on Gas-Chrom Q, 80-100 mesh, and operated isothermally at 130°C.

Field-desorption mass spectrometry

Mass spectra of the radiolabelled preparations of octan-2-sulphate were obtained by Dr D.E. Games, Chemistry Department, University College, Cardiff with a Varian-MAT CH5-D double-focusing instrument employed in the F.D. mode (6). The sulphate esters were prepared as potassium salts, but they were converted to sodium salts by ion-exchange chromatography (Dowex 50W, H^+ form) prior to mass-spectral analysis. Samples were dissolved in methanol:water (1:1, by vol.), and the spectra obtained at a wire current of 24mA.

Stereospecific enzymic hydrolysis

The stereochemical purity of the D(+)- and L(-)-octan-2-[^{35}S]sulphate was assessed by measuring the extent to which they were hydrolysed by the highly stereospecific CS2 enzyme of Comamonas terrigena. This secondary alkylsulphohydrolase only hydrolyses the D(+)-isomer of octan-2-sulphate (3,7). Only very small samples of the raiolabelled isomers were required for the enzymic assays, whereas much greater quantities would have been required for measurements of specific optical rotations (3).

For each isomer, 0.1ml of a 1.7mM aqueous solution of sulphate ester was mixed with 1.0ml of enzyme solution (2.3 units of enzyme (7)/ml 0.1M-Tris-maleate buffer, pH 7.5) and incubated at 31^{o}C. Samples of the incubation mixtures were removed at various times up to 21h and mixed with 1.0ml of trichloroacetic acid (40g/l). The percentage hydrolysis was determined by subjecting the acidified samples to paper electrophoresis (2h at a potential gradient of 7.5V/cm in 0.5M-pyridine/0.06M-acetic acid buffer, pH 6.0) and measuring the ratio of $^{35}SO_4^{2-}$ to [^{35}S]sulphate ester.

EXPERIMENTAL

Synthesis of DL-[2-^{14}C]Octan-2-ol

A Grignard reagent was prepared from 10mmol. of 1-bromohexane and subsequently carboxylated with $^{14}CO_2$ generated from 25mCi of $Ba^{14}CO_3$ (1.25mmol.). The radiochemical yield of [1-^{14}C]heptanioc acid was 80%.

The [1-^{14}C]heptanoic acid was methylated with an excess of diazomethane in ethereal solution (8). Reduction of the methyl ester with $LiAlH_4$ (5-fold molar excess) produced [1-^{14}C]heptan-1-ol in 90% yield, based on [1-^{14}C]heptanoic acid. When analysed by radio-g.l.c., the product was found to be approximately 98% radiochemically pure.

The ethereal solution of [1-^{14}C]heptan-1-ol was dried over anhydrous Na_2SO_4, unlabelled heptan-1-ol (2mmol.) was added and the ether removed under N_2. The [1-^{14}C]heptan-1-ol was redissolved in 3ml of sodium-dried toluene, and oxidized to [1-^{14}C]heptanal by refluxing with 1.2g of Seloxcette (equivalent to an approximately 2.5-fold molar excess of CrO_3) for 48h under dry N_2. A 25-33% conversion was obtained, based on radio-g.l.c. analysis. After cooling, the Seloxcette was filtered off and washed with toluene. The filtrate and washings were pooled. The [1-^{14}C]heptanal was separated from the remaining [1-^{14}C]heptan-1-ol by adsorption chromatography on a 25x1cm column of chromatographic grade silica gel (100-200 mesh) made up in light petroleum. Pure [1-^{14}C]heptanal was eluted with ether-light petroleum (1:9, by vol.), whilst unreacted [1-^{14}C]heptan-1-ol was recovered by eluting with ether. By carrying out two more cycles of oxidation of the unreacted alcohol followed by separation of unreacted alcohol, the yield of [1-^{14}C]heptanal from [1-^{14}C]heptan-1-ol was increased to 66%. The product was shown to be homogeneous by radio-g.l.c.

The [1-^{14}C]heptanal was diluted 2-fold with unlabelled heptanal, and then reacted with a 10-fold molar excess of methyl magnesium iodide to give DL-[2-^{14}C]octan-2-ol in a radiochemical yield of 43%. The overall radiochemical yield of DL-[2-^{14}C]octan-2-ol was 20%. Unlabelled DL-octan-2-ol was added so that the specific radioactivity of the alcohol was 0.45mCi/mmol. T.l.c. analysis showed that the product was 95% radiochemically pure.

Synthesis of DL-[2-^{14}C]Octyl-2-sulphate

DL-[2-^{14}C]Octan-2-ol was sulphated with pyridine-SO_3 adduct prepared by a modification of an established method (3): the pyridine and SO_3 were mixed in 1,2-dichloroethane instead of tetrachloromethane as it was found that the adduct prepared in the presence of 1,2-dichloroethane gave a 20% greater yield of sulphate ester. The sulphation procedure was a small-scale version of a method previously used to prepare unlabelled alkyl sulphates (3). The potassium DL-[2-^{14}C]octan-2-sulphate obtained (650mg) represented a radiochemical yield of 56% from DL-[2-^{14}C]octan-2-ol, and an overall radiochemical yield of 11%. It had a specific activity of 0.45mCi/mmol. T.l.c. analysis showed the product to be >99% radiochemically pure, and it gave an infra-red spectrum consistent with a secondary alkyl sulphate uncontaminated with alcohol (9). The major ion at $^{m}/e$ 487 obtained by field desorption mass spectrometry, corresponded to a (2M+Na) ion similar to those found with the sodium salts of other sulphate and sulphonate derivatives (4,6).

Synthesis of Pyridine-$^{35}SO_3$ Adduct

Pyridine-$^{35}SO_3$ was prepared by an adaptation of a method previously used to synthesize unlabelled adduct (10). The $^{35}SO_3$ (1.2g, 2.12mCi/mmol. on the day of synthesis) was dissolved in 15ml of 1,2-dichloroethane and cooled to -10°C. A mixture of pyridine (3ml) and 1,2-dichloroethane (10ml) was added

dropwise to the solution of $^{35}SO_3$ with stirring. The product was filtered off and dried in vacuo over P_2O_5. The adduct (1.4g) was obtained in 91% yield from $^{35}SO_3$. In order to obtain a maximum yield of sulphate ester, the pyridine-$^{35}SO_3$ was used within 1-3 days of preparation.

Synthesis of D(+)- and L(-)-Octan-2-[^{35}S]sulphate

D(+)- and L(-)-Octan-2-ol were sulphated with pyridine-$^{35}SO_3$ by the procedure used to sulphate DL-[2-^{14}C]octan-2-ol with unlabelled adduct. Approximately 400mg (40% radiochemical yield from alcohol) of each sulphate ester was obtained with a specific activity of 2.1mCi/mmol. on the day of synthesis. Both isomers were shown to be >99% radiochemically pure by t.l.c., and both gave an infra-red spectrum consistent with a secondary alkyl sulphate uncontaminated with alcohol (9). The base peaks in the field desorption spectra of the D(+)- and L(-)-isomers were $^{m}/e$ 719 [3M+Na] and $^{m}/e$ 255 [M+Na], respectively. The D(+)-isomer underwent 100% enzymic hydrolysis with the CS2 enzyme from C. terrigena whereas the L(-) isomer was hydrolysed only to an extent of 5% overall. These analyses by hydrolysis with the stereospecific alkyl-sulphohydrolase from C. terrigena confirm the stereochemical purity of the final ^{35}S-labelled products; the D(+)octan-2-[^{35}S]sulphate being 100% stereochemically pure and the L(-)-octan-2-[^{35}S]sulphate being 95% stereochemically pure.

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