BIOORGANIC CHEMISTRY 16, 283–297 (1988)

A Stereochemical Analysis of Chiral [^{16}O,^{17}O,^{18}O]Sulfate Monoesters Using Fourier Transform Infrared Spectroscopy

GORDON LOWE AND MARTIN J. PARRATT

Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford 0X1 3QY, England

Received January 26, 1988

The frequency shift caused by ^{17}O and ^{18}O on the symmetric and antisymmetric $>SO_2$ vibrational stretching modes of 2,2-dioxo-1,3,2-dioxathianes (six-membered-cyclic sulfate esters) is shown to be dependent on whether the oxygen isotope is located in an axial or equatorial site. This observation allows enantiomeric [^{16}O, ^{17}O, ^{18}O]sulfate monoesters of chiral 1,3-diols, such as (3*R*)-butane-1,3-diol, to be distinguished by infrared spectroscopy after cyclization to the isotopomeric mixture of (4*R*)-methyl-2,2-dioxo-1,3,2-dioxathianes.

INTRODUCTION

A general strategy has been developed for the synthesis of chiral [^{16}O, ^{17}O, ^{18}O]sulfate monoesters, with the goal of determining the stereochemical course of chemical- and enzyme-catalyzed sulfuryl transfer reactions (*1*). Although this method of synthesis gives [^{16}O, ^{17}O, ^{18}O]sulfate monoesters of known absolute configuration, a method of stereochemical analysis is required in order to determine the chirality of the product of a reaction. We now report a method for determining the chirality of [^{16}O, ^{17}O, ^{18}O]sulfate monoesters using Fourier transform infrared (FTIR)[1] spectroscopy.

RESULTS AND DISCUSSION

The frequency of an ir vibrational mode is markedly affected by isotopic substitution (*2*). Thus in sulfur dioxide substitution of one ^{16}O by ^{18}O leads to a shift of the symmetric and antisymmetric stretching modes by 30 and 19 cm^{-1}, respectively (*3*). In order to explore the conformational effect of ^{18}O on the symmetric and antisymmetric $>SO_2$ stretching modes in six-membered cyclic sulfate esters, 2,2-[^{18}O]dioxo-1,3,2-dioxathiane **1** was initially prepared from 2-oxo-1,3,2-dioxathiane by oxidation with ruthenium [$^{18}O_4$]tetroxide (*4*). Since this should exist as an equilibrium mixture of the chair conformations with ^{18}O in the axial **1a** and equatorial **1e** sites, it should provide a direct demonstration of the effect of confor-

[1] Abbreviation used: FTIR, Fourier transform infrared.

0045-2068/88 $3.00

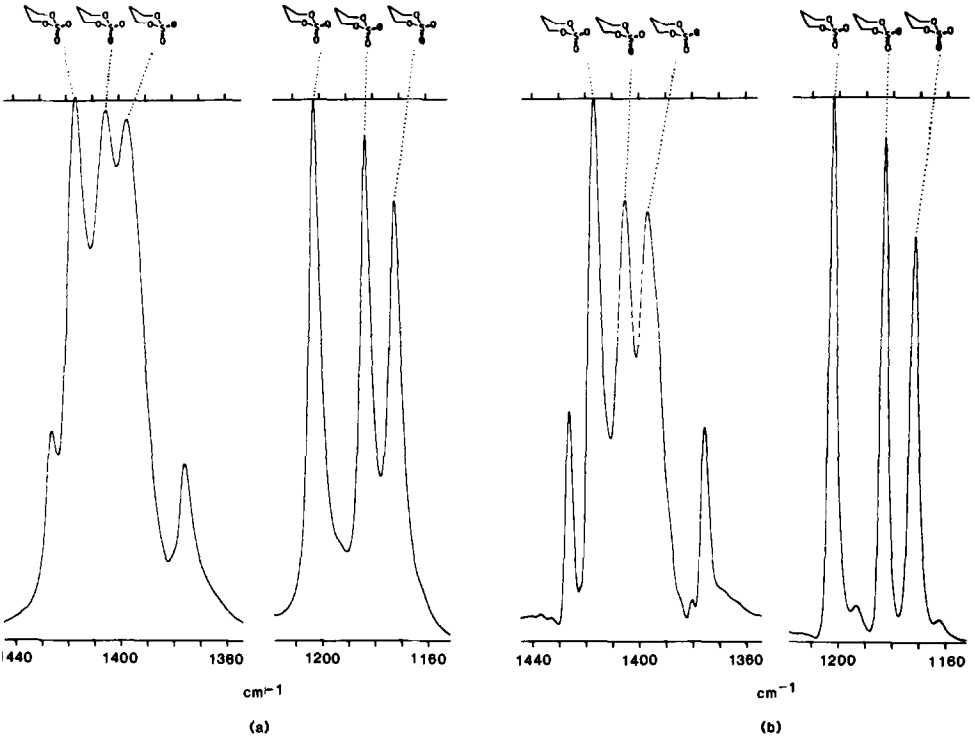


FIG. 1. (a) The FTIR spectrum of the antisymmetric and symmetric $>SO_2$ stretching region of 2,2-[^{18}O]dioxo-1,3,2-dioxathiane and (b) the same spectrum after deconvolution with an enhancement factor of 1.68 and linewidth at half height of 5 cm^{-1} for the antisymmetric $>SO_2$ stretching region and an enhancement factor of 1.50 and linewidth at half height of 4 cm^{-1} for the symmetric $>SO_2$ stretching region.

mation on the isotope shift of the $>SO_2$ symmetric and antisymmetric stretching frequencies.

The FTIR spectrum of **1** is shown in Fig. 1a (the ^{18}O site is only about 60% enriched). It is evident from this spectrum that the isotope shift on both the symmetric and antisymmetric modes of the $>SO_2$ group is conformationally dependent, but the isotope shift is greater and the linewidth narrower in the symmetric stretching mode. Separate deconvolution of these two spectral regions gave the resolution-enhanced spectrum shown in Fig. 1b. In order to assign the conformations responsible for the two symmetric and antisymmetric $>S$ [^{16}O, ^{18}O] stretching vibrations, the *cis*- and *trans*-cyclic sulfite esters **2** and **3**, derived from (3*R*)-butane-1,3-diol and thionyl chloride, were oxidized with ruthenium [$^{18}O_4$]tetroxide to give the isotopomeric cyclic [^{18}O]sulfate esters. Since this oxidation is known to proceed with retention of configuration at sulfur (*4*) the *cis*-sulfite **2** must give the (R_S)-[^{18}O]sulfate **4**, and the *trans*-sulfite **3** must give the (S_S)-[^{18}O]sulfate **5**. As expected, each isotopomer possesses only one symmetric and one antisymmetric $>SO_2$ stretching vibration (data in Table 1) since the confor-

TABLE 1

The Effect of Oxygen Isotopic Substitution on the Symmetric and Antisymmetric $>SO_2$ Stretching Frequencies of (4*R*)-Methyl-2,2-dioxo-1,3,2-dioxathianes

Isotope		Symmetric stretching frequency (cm^{-1})	Δ for symmetric stretching mode (cm^{-1})	Antisymmetric stretching frequency (cm^{-1})	Δ for antisymmetric stretching mode (cm^{-1})
Axial	Equatorial				
^{16}O	^{16}O	1201	—	1414	—
^{16}O	^{17}O	1192	9	1401	13
^{17}O	^{16}O	1186	15	1407	7
^{16}O	^{18}O	1183	18	1392	22
^{18}O	^{16}O	1172	29	1401	13
^{17}O	^{18}O	1170	31	1384	30
^{18}O	^{17}O	1163	38	1389	25
^{18}O	^{18}O	1157	44	1378	36

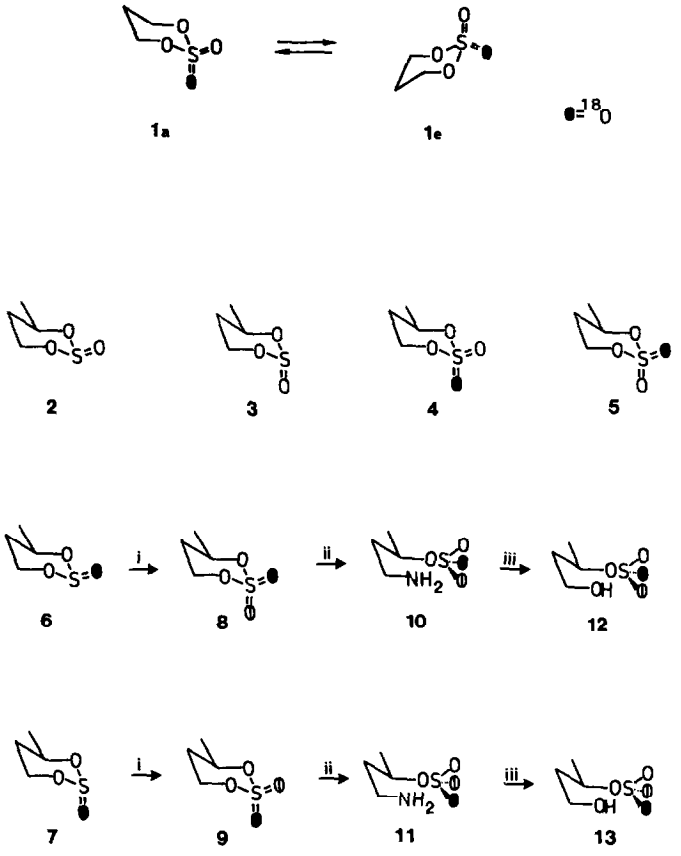
Note. Δ is the isotope shift from the $>S^{16}O_2$ frequency.

mation with the methyl group equatorial is strongly preferred. The values differ slightly from those observed for 2,2-[^{18}O]dioxo-1,3,2-dioxathiane **1** (by about 1 cm^{-1}), but allow the assignments to be made unambiguously as shown in Fig. 1.

The *cis*- and *trans*-cyclic [^{18}O]sulfite esters **6** and **7** have also been prepared from (3*R*)-butane-1,3-diol and [^{18}O]thionyl chloride. Oxidation of these and the cyclic sulfite esters **2** and **3** with ruthenium [$^{16}O_4$]-, [$^{17}O_4$]-, or [$^{18}O_4$]tetroxide allowed eight isotopomers to be prepared and the frequency of their symmetric and antisymmetric stretching vibrations to be determined. These are shown in Table 1 together with the isotope shift from the $>SO_2$ symmetric and antisymmetric vibrational frequencies. As expected the isotope shift caused by ^{18}O is about twice the isotope shift caused by ^{17}O, and the isotope shifts are approximately additive. The difference in the isotope shift observed when the heavy oxygen isotope is in the axial compared with the equatorial position is gratifyingly large. Moreover, since each of the diastereoisotopomeric pairs are easily distinguishable, especially in the symmetric stretching mode, ir spectroscopy should provide a means for distinguishing between enantiomeric [^{16}O, ^{17}O, ^{18}O]sulfate monoesters.

In order to establish the method for the stereochemical analysis of chiral [^{16}O, ^{17}O, ^{18}O]sulfate monoesters based on FTIR spectroscopy (S_S)- and (R_S)-3(*R*)-butan-1-ol-3[^{16}O, ^{17}O, ^{18}O]sulfate were prepared as outlined in Scheme 1. *cis*- and *trans*-4(*R*)-Methyl-2-[^{18}O]oxo-1,3,2-dioxathianes **6** and **7** were oxidized separately with ruthenium [$^{17}O_4$]tetroxide (prepared *in situ* from ruthenium dioxide, sodium periodate, and ^{17}O-water) (*4*), giving 4(*R*)-methyl-2[(*S*)-^{17}O, ^{18}O]dioxo-1,3,2-dioxathiane **8** and 4(*R*)-methyl-2[(*R*)-^{17}O, ^{18}O]dioxo-1,3,2-dioxathiane **9**, respectively.

The hydrolytic cleavage of 4-methyl-2,2-dioxo-1,3,2-dioxathiane has been extensively studied, but no conditions were found which gave exclusive cleavage of

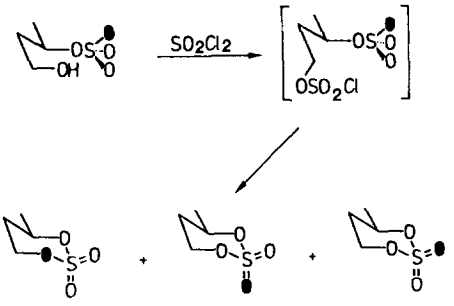


SCHEME 1. The synthesis of (S_s)- and (R_s)-[(^{16}O, ^{17}O, ^{18}O]sulfate esters. Reagents: i, $Ru^{17}O_4$ (from RuO_2, $NaIO_4$, and $H_2{}^{17}O$); ii, NH_3,MeOH; iii, $NaNO_2$, aq AcOH. ⦶, ^{17}O; ●, ^{18}O.

the primary C–O bond (5). Ammonia in methanol, however, gave the desired mode of ring cleavage, the primary amines **10** and **11** being isolated virtually quantitatively. The corresponding primary alcohols **12** and **13** were obtained by treatment of the amines with nitrous acid in 83% yield.

It was necessary to develop a stereospecific method for the cyclization of the enantiomeric [^{16}O, ^{17}O, ^{18}O]sulfate monoesters **12** and **13.** Lack of precedent for the formation of cyclic sulfate esters from acyclic sulfate monoesters led to the exploration of several possible reagents. Only two were found: namely, trifluoromethanesulfonic anhydride and sulfuryl chloride; the latter gave better yields and has been used throughout.

In order to investigate whether there was any isotope exchange during cyclization, 3(*R*)-butan-1-ol-3[^{18}O]sulfate was prepared from **5** and cyclized with sulfuryl chloride. The ammonia chemical ionization mass spectrum of the cyclic sulfate revealed a molecular ion at *m/z* 172 only (M_r for $C_4H_8SO_4 \cdot NH_4^+$ is 170 and $C_4H_8SO_3{}^{18}O \cdot NH_4^+$ is 172), suggesting that cyclization had occurred by activation



SCHEME 2. The mechanism of cyclization of (1*R*)-3-hydroxymethylpropyl sulfate.

of the primary alcohol followed by intramolecular displacement by the sulfate monoester (Scheme 2). This mode of cyclization was confirmed by the natural abundance ^{13}C NMR spectrum of the cyclic sulfate which showed C-1 to be split into two resonances at 71.784 and 71.749 ppm, the endocyclic ^{18}O causing an upfield shift of 0.035 pm as expected (*6*), and in a 2:1 ratio of intensity after correcting for the ^{18}O enrichment of the sulfate monoester; thus no loss of isotope had occurred. It was of interest to investigate the FTIR spectrum of the mixture of isotopomeric cyclic sulfate esters. As expected three absorption bands were observed in both the symmetric and antisymmetric $>SO_2$ stretching regions (Fig. 2). For the isotopomer containing ^{18}O in the C–O–S bridge the symmetric and antisymmetric $>SO_2$ absorption bands were at 1201 and 1414 cm^{-1}, respectively, i.e., identical (at 1 cm^{-1} resolution) to those for 4(*R*)-methyl-2,2-dioxo-1,3,2-dioxathiane (and consequently not resolved from a small amount of unlabeled material). Thus a heavy oxygen isotope in the C–O–S bridge of the cyclic sulfate ester leaves both the symmetric and antisymmetric $>SO_2$ stretching frequencies unperturbed.

Since none of the S–O bonds are broken in the cyclization of 3(*R*)-butan-1-ol-3-sulfate with sulfuryl chloride the cyclization should proceed stereospecifically for a chiral [^{16}O, ^{17}O, ^{18}O]sulfate with retention of configuration. In order to confirm this prediction the [(*S*)-^{16}O, ^{17}O, ^{18}O]sulfate ester **12** and the [(*R*)-^{16}O, ^{17}O, ^{18}O]sulfate ester **13** were cyclized with sulfuryl chloride and the FTIR spectra of the isotopomeric mixture of cyclic sulfate esters measured. The spectra of the symmetric and antisymmetric $>SO_2$ stretching vibrations are shown in Fig. 3.

Scheme 3 shows the mixture of isotopomeric 4(*R*)-methyl-2,2-dioxo-1,3,2-dioxathianes that should be formed by cyclizing the (S_s)- and (R_s)-chiral [^{16}O, ^{17}O, ^{18}O]sulfate esters **12** and **13** with retention of configuration at sulfur by the mechanism outlined in Scheme 2. If all three isotopes were fully enriched only the three isotopomers shown on the top row of each set would be obtained, but in practice the "^{17}O-site" consists of a substantial amount of ^{16}O and ^{18}O, and therefore nine isotopomeric species should be formed; the ^{18}O site is 99 atom% ^{18}O. The symmetric and antisymmetric $>SO_2$ stretching frequencies are shown for each isotopomer.

The spectra shown in Figs. 3a and 3b are easily distinguishable. This is because

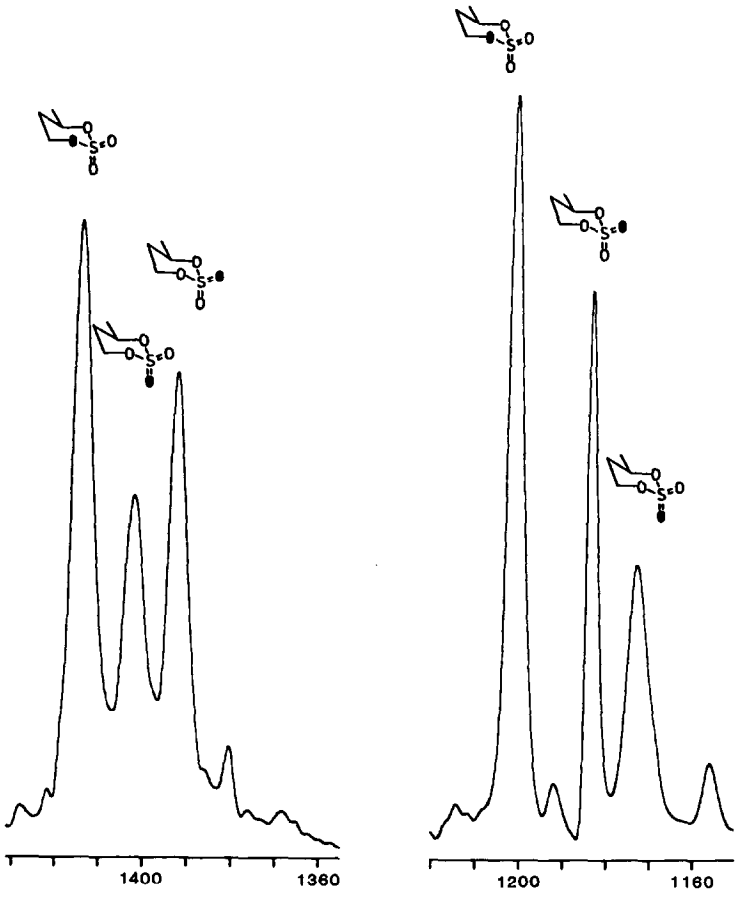
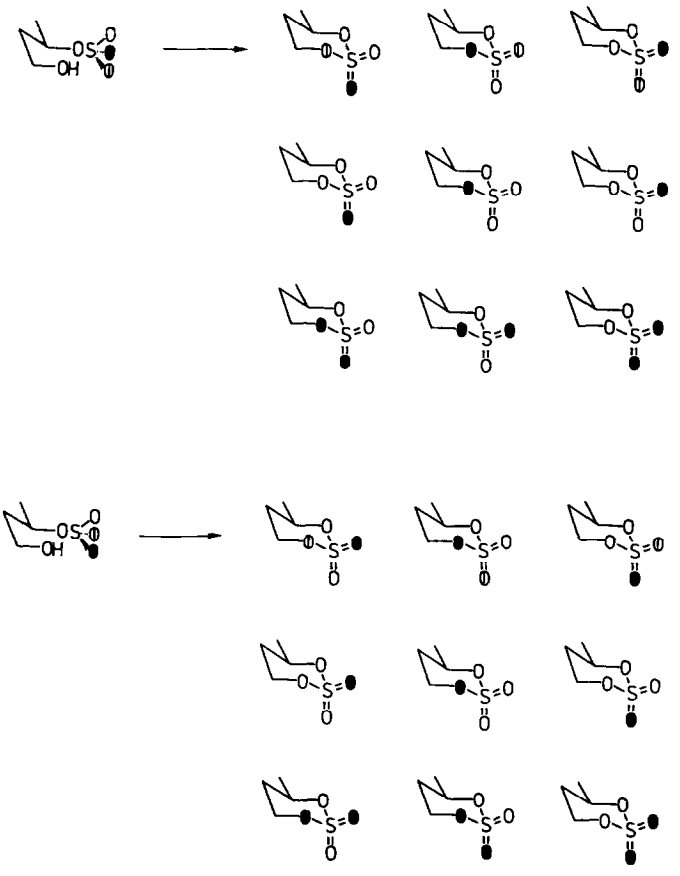


FIG. 2. The FTIR spectrum showing the symmetric and antisymmetric $>SO_2$ stretching frequencies of the isotopomeric mixture obtained by cyclizing 3(*R*)-butan-1-ol-3[^{18}O]sulfate with sulfuryl chloride. The spectral resolution was enhanced by Fourier deconvolution: for the symmetric stretching region a linewidth of 12 cm^{-1} and an enhancement factor of 1.5 were used, whereas for the antisymmetric stretching region a linewidth of 20 cm^{-1} and an enhancement factor of 2.0 were used. The symmetric and antisymmetric $>SO_2$ stretching frequencies at 1201 and 1414 cm^{-1}, respectively, coincide with those for unlabeled 4(*R*)-methyl-2,2-dioxo-1,3,2-dioxathiane. The scale is in cm^{-1}. ●, ^{18}O.

the peaks for the [$^{17}O_{ax}$, $^{16}O_{eq}$]- and [$^{18}O_{ax}$, $^{17}O_{eq}$]-isotopomers are absent in Fig. 3a and present in Fig. 3b, whereas the peaks due to the [$^{16}O_{ax}$, $^{17}O_{eq}$]- and [$^{17}O_{ax}$, $^{18}O_{eq}$)-isotopomers are present in Fig. 3a and absent in Fig. 3b. In addition the ratio of the intensities of the peaks for the [$^{18}O_{ax}$, $^{16}O_{eq}$]- and [$^{16}O_{ax}$, $^{18}O_{eq}$]-isotopomers are different in the two spectra.

It should be noted that the linewidth and extinction coefficient for different isotopomers are significantly different. This is well illustrated in Fig. 2 where the [$^{18}O_{ax}$, $^{16}O_{eq}$)- and [$^{16}O_{ax}$, $^{18}O_{eq}$]-isotopomers must be present in equimolar amounts. If peak intensities are used to quantify the stereochemical analysis this



SCHEME 3. The cyclization of (S_s)- and (R_s)-[^{16}O, ^{17}O, ^{18}O]sulfate esters **12** and **13** with retention of configuration at sulfur. If the three isotopes were fully enriched only the three isotopomers shown in the top row of each set would be formed, but since the "^{17}O-site" contains substantial amounts of ^{16}O and ^{18}O, nine isotopomers will be formed as shown from each chiral sulfate. ⊕, ^{17}O; ●, ^{18}O.

fact must be taken into account. It is much simpler and more accurate to look for the presence and absence of the isotopomers containing ^{17}O in the symmetric $>SO_2$ stretching frequency region of the spectrum. In the set of isotopomers derived from the chiral [(*S*)-^{16}O, ^{17}O, ^{18}O]sulfate ester **12** the [$^{16}O_{ax}$, $^{17}O_{eq}$]-isotopomer (1192 cm^{-1}) should be well resolved, whereas the [$^{17}O_{ax}$, $^{18}O_{eq}$] will not. In the set of isotopomers derived from the chiral [(*R*)-^{16}O, ^{17}O, ^{18}O]sulfate ester **13,** the [$^{18}O_{ax}$, $^{17}O_{eq}$]-isotopomer (1163 cm^{-1}) should be well resolved, whereas the [$^{17}O_{ax}$, $^{16}O_{eq}$]-isotopomer (1186 cm^{-1}) should be only partially resolved. Thus the enantiomeric excess can be calculated from the absorbance at 1192 and 1163 cm^{-1} after taking into account their relative extinction coefficients. In practice the analysis becomes more accurate by subtracting out the spectra of other isotopomers that contain weak bands at these frequencies. For example the [^{16}O, ^{16}O]-isotopomer shows a weak band at 1192 cm^{-1} (see Fig. 2) which interferes with the quantitative

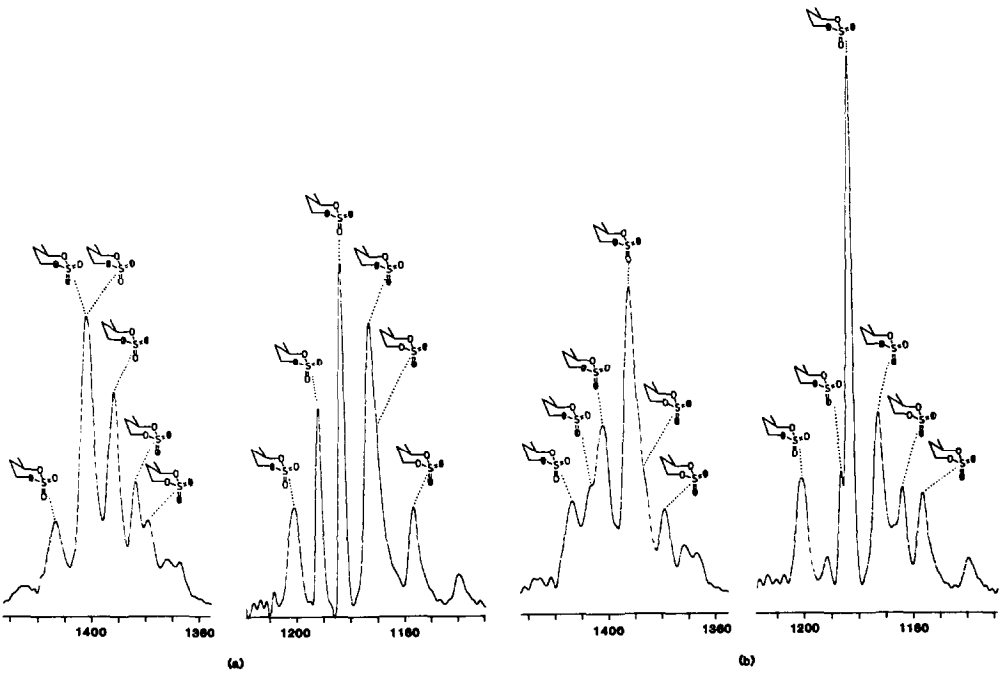


FIG. 3. The FTIR spectra showing the symmetric and antisymmetric $\geq SO_2$ stretching frequencies of the isotopomeric mixture obtained by cyclizing, with sulfuryl chloride. (a) 3(*R*)-butan-1-ol-3[(*S*)-^{16}O, ^{17}O, ^{18}O]sulfate **12** in which the "^{17}O-site" consists of 37.4 atom% ^{16}O, 36.4 atom% ^{17}O, and 26.2 atom% ^{18}O and (b) 3(*R*)-butan-1-ol-3[(*R*)-^{16}O, ^{17}O, ^{18}O]sulfate **13** in which the "^{17}O-site" consists of 36.0 atom% ^{16}O, 37.1 atom% ^{17}O, and 26.9 atom% ^{18}O. The spectra were determined as described in the legend to Fig. 2. The scale is in cm^{-1}. ⊕, ^{17}O; ●, ^{18}O; ◑, $^{16}O + {}^{18}O$; ⊗, $^{16}O + {}^{17}O + {}^{18}O$.

analysis of the [$^{16}O_{ax}$, $^{17}O_{eq}$]-isotopomer, but this can easily be removed by subtracting the spectrum of the [^{16}O, ^{16}O]-isotopomer until the symmetrical $\geq SO_2$ stretching frequency at 1201 cm^{-1} has been removed. As expected the cyclization occurs stereospecifically within this experimental limit.

EXPERIMENTAL PROCEDURES

All solvents and reagents were purified and dried by conventional procedures. Melting points were determined using a Kofler block and are uncorrected. Mass spectra were recorded on a VG 16F mass spectrometer. Proton magnetic resonance spectra were recorded on a Bruker AM 500 Fourier transform spectrometer at 500.13 MHz. ^{13}C NMR spectra were recorded on the same spectrometer at 125.77 MHz using a ^{13}C dedicated probe using broadband proton decoupling. All chemical shifts are reported as positive for resonances downfield of the reference signal of external tetramethylsilane. The infrared spectra were recorded on a Perkin–Elmer 1750 Fourier transform infrared spectrometer with a Perkin–Elmer 7300 professional computer at a resolution of 1 cm^{-1}.

(3*R*)-Butane-1,3-diol (Aldrich), $[\alpha]_D^{20}$ −22.05° (*c* 1, EtOH) contains 15% of the (3*S*)-enantiomer as determined by the method of Anderson and Shapiro (*7*). The highest recorded optical rotation of (3*R*)-butane-1,3-diol is $[\alpha]_D^{20}$ −31.6° (*c* 1, EtOH) (*8*).

2-Oxo-1,3,2-dioxathiane. 1,3-Propanediol (500 mg, 6.58 mmol) was dissolved in dry ether (23 ml) containing dry pyridine (1.15 mol, 14.2 mmol). To this solution stirred at 0°C was added dropwise a solution of thionyl chloride (0.52 ml, 7.12 mmol) in ether (8 ml). The mixture was stirred at room temperature for 1 h and then extracted with saturated sodium bicarbonate solution (8 ml) and brine (8 ml). The ether solution was dried ($MgSO_4$), filtered, and evaporated to leave the product (350 mg), R_f (ether) 0.20, ν_{max} (SO) 1195 cm^{-1}, δ_H ($CDCl_3$) 1.63 (1H, d quintet, 5-H_e, $2J_{HH}$ 15 Hz, $^3J_{HH}$ 2 Hz), 2.50 (1H, m, 5-H_a), 3.86 (2H, m, 4-H_e + 6 − H_e), 4.93 (2H, m, $4H_a$ + $6H_a$).

2,2-[^{18}O]Dioxo-1,3,2-dioxathiane ***1.*** Sodium periodate (93 mg, 0.44 mmol) was stirred in ^{18}O-water (50 μl) for 5 min and then ethanol-free chloroform (chloroform distilled from phosphorus pentoxide) (0.75 ml) was added. Ruthenium dioxide hydrate (32 mg, 0.19 mmol) was added and the mixture stirred *rapidly* until it had changed from black to a pale green solution (15 min). The solution was cooled to 0°C, then a solution of 2-oxo-1,3,2-dioxathiane (19.6 mg, 0.16 mmol) in chloroform (distilled from P_2O_5) (0.5 ml) was added. The mixture was allowed to warm to room temperature and stirred for 10 min before the reaction was quenched by addition of isopropanol (1 ml). The mixture was diluted with chloroform (10 ml) and filtered. The filtrate was dried ($MgSO_4$) and evaporated to leave a yellow oil. Flash chromatography with ether as eluant gave the title compound (8 mg, 36%) as a colorless oil, R_f (ether) 0.25, which recrystallized from ether–hexane, mp 62°C. δ_H ($CDCl_3$) 2.15 (2H, quintet, $^3J_{HH}$ 6 Hz), 4.75 (4H, t, $^3J_{HH}$ 6 Hz) ν_{max} ($S^{16}O_2$, symmetric) 1202 cm^{-1}, ($S^{16}O_e{}^{18}O_a$, symmetric) 1172 cm^{-1}, ($S^{16}O_a{}^{18}O_e$, symmetric) 1184 cm^{-1}, ($S^{16}O_2$, antisymmetric) 1417 cm^{-1}, ($S^{16}O_e{}^{18}O_a$ antisymmetric) 1405 cm^{-1}, ($S^{16}O_a{}^{18}O_e$, antisymmetric) 1397 cm^{-1}.

2,2-[^{17}O]Dioxo-1,3,2-dioxathiane. Sodium periodate (186 mg, 0.88 mmol) was stirred in ^{17}O-water (100 μl) for 5 min and then ethanol-free chloroform (1.5 ml) added. Ruthenium dioxide hydrate (10 mg, 0.06 mmol) was added and the mixture stirred rapidly to give a pale green solution. A solution of 2-oxo-1,3,2-dioxathiane (38 mg, 0.32 mmol) in chloroform (0.5 ml) was added to the solution at room temperature. The mixture was stirred until the green color reappeared (1 h) and then quenched with isopropanol (1 ml). The mixture was filtered, and the filtrate dried ($MgSO_4$) and evaporated to leave an oil. Flash chromatography with ether as eluant gave the title compound as a colorless oil (20 mg, 45%), R_f (ether) 0.25, which crystallized from ether–hexane, mp 62°C. δ_H ($CDCl_3$) 2.15 (2H, quintet, $^3J_{HH}$ 6 Hz), 4.75 (4H, t, $^3J_{HH}$ 6 Hz); ν_{max} [symmetric; $S^{16}O_2$ 1202, $S^{16}O_a{}^{17}O_e$ 1193, $S^{16}O_e{}^{17}O_a$ 1187, $S^{16}O_a{}^{18}O_e$ 1184, $S^{16}O_e{}^{18}O_a$ 1172 cm^{-1}], [antisymmetric; $S^{16}O_2$ 1417, $S^{16}O_e{}^{17}O_a$ 1411, $S^{16}O_a{}^{17}O_e$ 1406, $S^{16}O_e{}^{18}O_a$ 1406, $S^{16}O_a{}^{18}O_e$ 1397 cm^{-1}].

cis- and trans-(4R)-Methyl-2-oxo-1,3,2-dioxathianes ***2*** *and* ***3.*** (*R*)-1,3-Butanediol (257 mg, 2.85 mmol) was dissolved in dry ether (10 ml) containing pyridine (0.5 ml, 6.18 mol). The mixture was cooled to 0°C, and a solution of thionyl chloride (370 mg, 3.11 mmol) in dry ether (3.5 ml) added dropwise. The mixture was stirred at

room temperature for 1 h and then extracted with saturated sodium bicarbonate solution (2.5 ml) and brine (2.5 ml). The ether layer was dried ($MgSO_4$), filtered, and evaporated to give a colorless oil (200 mg). The *cis*- and *trans*-isomers of the title compound were separated by flash chromatography by elution with ether–hexane (v/v, 1 : 1) to give two fractions, (a) the *trans*-isomer **3** (117.5 mg, 30%), R_f (ether–hexane, 1 : 1) 0.44, δ_H ($CDCl_3$) 1.29 (3H, d, *Me*, $^3J_{HH}$ 6.3 Hz), 1.69 (1H, dq, 5-*H*, $^2J_{HH}$ 14.3 Hz, $^3J_{HH}$ 2.2 Hz), 2.19 (1H, m, 5-*H*), 3.89 (1H, m, 6-*H*), 4.97 (1H, m, 6-*H*), 5.10 (1H, m, 4-*H*); δ_C ($CDCl_3$) 21.25 (q, CH_3), 33.39 (t, *C*-5) 57.47 (t, *C*-6), 64.15 (d, *C*-4); ν_{max} (SO_a) 1194 cm^{-1}, $[\alpha]_D$ −12.35 (*c* 1, $CHCl_3$); and (b) The *cis*-isomer **2** (72.5 mg, 19%), R_f (ether–hexane 1 : 1) 0.23, δ_H ($CDCl_3$) 1.47 (3H, d, *Me*, $^3J_{HH}$ 6.3 Hz), 1.77 (1H, dq, 5-*H*, $^2J_{HH}$ 14.2 Hz, $^3J_{HH}$ 3.4 Hz), 1.99 (1H, m, 5-*H*), 4.35 (1H, m, 6-*H*), 4.52 (1H, m, 6-*H*), 4.59 (1H, m, 4-*H*); δ_C ($CDCl_3$) 21.39 (q, CH_3), 31.11 (t, *C*-5), 63.48 (t, *C*-6), 74.11 (d, *C*-4); ν_{max} (SO_e) 1247 cm^{-1}, (SO_a) 1196 cm^{-1}; $[\alpha]_D$ −1.68 (*c* 1, $CHCl_3$).

[^{18}O]*Thionyl chloride*. Phosphorus pentachloride (1.11 g, 5.32 mmol) was placed in a Carius tube and the tube evacuated. [$^{18}O_2$]sulfur dioxide (250 μl, 5.32 mmol) was transferred into the Carius tube (cooled in liquid N_2) attached to a vacuum line and the tube sealed. The mixture was left in the tube at room temperature for 2 days and then opened and the liquid used without purification.

cis- and trans-(4R)-4-Methyl-2-[^{18}O]*oxo-1,3,2-dioxathianes* ***6*** *and* ***7***. The crude mixture of [^{18}O]thionyl chloride (5.32 mmol) and phosphorus [^{18}O]oxychloride (5.32 mmol) was dissolved in dry ether (20 ml) and added dropwise to a solution of (*R*)-1,3-butanediol (0.96 g, 10.64 mmol) in dry ether (70 ml) at 0°C containing dry pyridine (2.15 ml, 26.6 mol). The mixture was stirred at room temperature for 15 min and then extracted with saturated sodium bicarbonate solution (8 ml) and brine (8 ml). The ether layer was dried ($MgSO_4$), filtered, and evaporated to leave an oil. Flash chromatography using ether–hexane (v/v, 1 : 1) as eluant gave the *cis*- and *trans*-isomers of the title compound as two fractions: (a) the *trans*-isomer (137 mg, 19%), R_f (ether–hexane, 1 : 1) 0.44, δ_H ($CDCl_3$) 1.29 (3H, d, *Me*, $^3J_{HH}$ 6.3 Hz), 1.69 (1H, dq, 5-*H*, $^2J_{HH}$ 14.3 Hz, $^3J_{HH}$ 2.2 Hz), 2.19 (1H, m, 5-*H*), 3.89(1H, m, 6-*H*), 4.97 (1H, m, 6-*H*), 5.10 (1H, m, 4-*H*); ν_{max} ($S^{18}O_a$) 1159 cm^{-1}; (b) the *cis*-isomer (157 mg, 21%), R_f (ether–hexane, 1 : 1) 0.23, δ_H ($CDCl_3$) 1.47 (3H, d, *Me*, $^3J_{HH}$ 6.3 H), 1.77 (1H, dq, 5-*H*, $^2J_{HH}$ 14.2 Hz, $^3J_{HH}$ 3.4 Hz), 1.99 (1H, m, 5-*H*), 4.35 (1H, m, 6-*H*), 4.52 (1H, m, 6-*H*), 4.59 (1H, m, 4-*H*); ν_{max} ($S^{18}O_e$) 1204 cm^{-1}, ($S^{18}O_a$) 1159 cm^{-1}.

(4R)-Methyl-2,2-dioxo-1,3,2-dioxathiane. Sodium periodate (0.74 g, 3.5 mmol) was added to water (1 ml) and then chloroform (6 ml) added. To the rapidly stirred mixture was added ruthenium dioxide hydrate (36 mg, 0.21 mmol). After the mixture had turned pale green, a solution of (4*R*)-methyl-2-oxo-1,3,2-dioxathiane (0.22 g, 1.62 mmol) in chloroform (1 ml) was added. The mixture was stirred rapidly until it turned green again (1 h) and then quenched with isopropanol (1 ml). The mixture was filtered, and the filtrate dried ($MgSO_4$) and evaporated to leave an oil. Flash chromatography using ether as eluant gave the title compound (188 mg, 70%) as a colorless oil, R_f (ether) 0.35, δ_H ($CDCl_3$) 1.49 (3H, d, *Me*, $^3J_{HH}$ 6.3 Hz), 1.88 (1H, m, 5-*H*), 2.17 (1H, m, 5-*H*), 4.58 (1H, m, 6-H_e), 4.80 (1H, m, 6-H_a), 5.07 (1H, m, 4-*H*); ν_{max} ($S^{16}O_2$, symmetric) 1201 cm^{-1}, ($S^{16}O_2$, antisymmetric) 1414

cm^{-1}; *m/z* 170 (NH_3 C.I.); $C_4H_8SO_4 \cdot NH_4^+$ requires *m/z* 170; $[\alpha]_D$ + 1.15 (*c* 1, $CHCl_3$).

(4R)-Methyl-2(S)-2,2[^{17}O]dioxo-1,3,2-dioxathiane. Sodium periodate (86 mg, 0.40 mmol) was stirred in ^{17}O-water (50 μl) for 5 min and then chloroform (1 ml) added. To the rapidly stirred mixture was added ruthenium dioxide hydrate (30 mg, 0.17 mmol). When the solution was pale green it was cooled to 0°C and a solution of *trans*(4*R*)-methyl-2-oxo-1,3,2-dioxathiane **3** (17.5 mg, 0.13 mmol) in chloroform (0.5 ml) added. The mixture was allowed to warm to room temperature, stirred for 10 min, and quenched with isopropanol (1 ml). The mixture was diluted with chloroform (10 ml) and filtered. The filtrate was dried ($MgSO_4$) and evaporated to leave an oil. Flash chromatography (eluting with ether) gave the title compound as a colorless liquid (19 mg, 96%), R_f (ether) 0.35, ν_{max} [symmetric; $S^{16}O_2$ 1201, $S^{16}O_a{}^{17}O_e$ 1192, $S^{16}O_a{}^{18}O_e$ 1183 cm^{-1}], [antisymmetric; $S^{16}O_2$ 1414, $S^{16}O_a{}^{17}O_e$ 1401, $S^{16}O_a{}^{18}O_e$ 1392 cm^{-1}]; *m/z* (NH_3 C.I.) 170 (38.0%), 171 (35.6%), 172 (26.4%); $C_4H_8SO_3{}^{17}O \cdot NH_4^+$ requires *m/z* 171.

(4R)-Methyl-(2R)-2,2-[^{17}O]dioxo-1,3,2-dioxathiane. To a solution of ruthenium [$^{17}O_4$]tetraoxide (0.17 mmol) prepared as above was added a solution of *cis*-(4*R*)-methyl-2-oxo-1,3,2-dioxathiane **2** (17.5 mg, 0.13 mmol) in chloroform (0.5 ml). Workup as above gave the title compound as a colorless liquid (17 mg, 86%), R_f (ether) 0.35, ν_{max} [symmetric; $S^{16}O_2$ 1201, $S^{16}O_e{}^{17}O_a$ 1186, $S^{16}O_a{}^{18}O_a$ 1172 cm^{-1}], [antisymmetric; $S^{16}O_2$ 1414, $S^{16}O_e{}^{17}O_a$ 1407, $S^{16}O_e{}^{18}O_a$ 1401 cm^{-1}]; *m/z* (NH_3 C.I.) 170 (44.1%), 171 (32.7%), 172 (23.2%); $C_4H_8SO_3{}^{17}O \cdot NH_4^+$ requires *m/z* 171.

(4R)-Methyl-(2S)-2,2-[^{18}O]dioxo-1,3,2-dioxathiane ***5.*** To a solution of ruthenium [$^{18}O_4$]tetraoxide (0.19 mmol) prepared as above was added a solution of *trans*-(4*R*)-methyl-2-oxo-1,3,2-dioxathiane **3** (22 mg, 0.16 mmol) in chloroform (0.5 ml). Workup as before gave the title compound as a colorless oil (19 mg, 77%), R_f (ether) 0.35, ν_{max} [symmetric; $S^{16}O_2$ 1201, $S^{16}O_a{}^{18}O_e$ 1183 cm^{-1}], [antisymmetric; $S^{16}O_2$ 1414, $S^{16}O_a{}^{18}O_e$ 1392]; *m/z* (NH_3 C.I.) 170 (24.5%), 172 (75.5%); $C_4H_8SO_3{}^{18}O \cdot NH_4^+$ requires *m/z* 172.

(4R)-Methyl-(2R)-2,2-[^{18}O]dioxo-1,3,2-dioxathiane ***4.*** To a solution of ruthenium [$^{18}O_4$]tetraoxide (0.19 mmol) prepared as above was added a solution of *cis*-(4*R*)-methyl-2-oxo-1,3,2-dioxathiane **2** (22 mg, 0.16 mmol) in chloroform (0.5 ml). Workup as before gave the title compound as a colorless oil (20 mg, 81%), R_f (ether) 0.35, ν_{max} [symmetric $S^{16}O_2$ 1201, $S^{16}O_e{}^{18}O_a$ 1172 cm^{-1}], [antisymmetric; $S^{16}O_2$ 1414, $S^{16}O_e{}^{18}O_a$ 1401 cm^{-1}]; *m/z* (NH_3 C.I.) 170 (37.7%), 172 (62.3%), $C_4H_8SO_3{}^{18}O \cdot NH_4^+$ requires *m/z* 172.

(4R)-Methyl-(2S)-2,2-[^{17}O, ^{18}O]dioxo-1,3,2-dioxathiane ***8.*** To a solution of ruthenium [$^{17}O_4$]tetraoxide (52 μmol) produced as above was added a solution of *cis*-(4*R*)-methyl-2-[^{18}O]oxo-1,3,2-dioxathiane **6** (6 mg, 44 μmol) in chloroform (0.2 ml). Workup as above gave the title compound as a colorless oil (4 mg 59%), R_f (ether) 0.35, ν_{max} [symmetric; $S^{16}O_a{}^{18}O_e$ 1183, $S^{17}O_a{}^{18}O_e$ 1170, $S^{18}O_2$ 1157 cm^{-1}], [antisymmetric; $S^{16}O_a{}^{18}O_e$ 1392, $S^{17}O_a{}^{18}O_e$ 1384, $S^{18}O_2$ 1378 cm^{-1}].

Use of a catalytic amount of ruthenium dioxide. Sodium periodate (210 mg, 0.98 mmol) was stirred with ^{17}O-water (133 μl) for 5 min and then chloroform (2 ml) added. To a rapidly stirred mixture was added ruthenium dioxide hydrate (7 mg, 40 μmol). After the mixture had turned pale green, a solution of *cis* (4*R*)-methyl-2-

[^{18}O]oxo-1,3,2-dioxathiane **6** (50 mg, 0.36 mmol) in chloroform (1 ml) was added. Rapid stirring was continued until the green color returned (1 h) and then the reaction was quenched with isopropanol (1 ml). Workup as above gave the title compound as a colorless oil (44 mg, 79%), R_f (ether) 0.35, ν_{max} [symmetric $S^{16}O_a{}^{18}O_e$ 1183, $S^{17}O_a{}^{18}O_e$ 1170, $S^{18}O_2$ 1157 cm^{-1}], [antisymmetric; $S^{16}O_a{}^{18}O_e$ 1392, $S^{17}O_a{}^{18}O_e$ 1384, $S^{18}O_2$ 1378 cm^{-1}]; *m/z* (NH_3 C.I.) 172 (34.7%), 173 (35.8%), 174 (29.5%); $C_4H_8SO_2{}^{17}O^{18}O \cdot NH_4^+$ requires *m/z* 173.

(4R)-Methyl-(2R)-2,2,-[^{17}O, ^{18}O]dioxo-1,3,2-dioxathiane **9**. To a solution of ruthenium [$^{17}O_4$]tetraoxide (0.25 mmol) prepared as above, was added a solution of *trans*-(4*R*)-methyl-2-[^{18}O]oxo-1,3,2-dioxathiane **7** (29 mg, 0.21 mmol) in chloroform (1 ml). Work up as above gave the title compound as a colorless oil (20 mg, 61%), R_f (ether) 0.35, ν_{max} [symmetric $S^{16}O_e{}^{18}O_a$ 1172, $S^{17}O_e{}^{18}O_a$ 1163, $S^{18}O_2$ 1157 cm^{-1}], [antisymmetric; $S^{16}O_e{}^{18}O_a$ 1401, $S^{17}O_e{}^{18}O_a$ 1389, $S^{18}O_2$ 1378 cm^{-1}].

Use of a catalytic amount of ruthenium dioxide. Sodium periodate (304 mg, 1.42 mmol) was stirred with ^{17}O-water (152 μl) for 5 min and then chloroform (2 ml) was added. To the rapidly stirred mixture was added ruthenium dioxide hydrate (24 mg, 0.14 mmol). After the mixture had turned pale green, a solution of *trans*-(4*R*)-methyl-2-[^{18}O]oxo-1,3,2-dioxathiane **7** (67 mg, 0.49 mmol) in chloroform (2 ml) was added. Rapid stirring was continued until the green color returned (1 h). Workup as above gave the title compound as a colorless oil (67 mg, 88%), R_f (ether) 0.35, ν_{max} [symmetric $S^{16}O_e{}^{18}O_a$ 1172, $S^{17}O_e{}^{18}O_a$ 1163, $S^{18}O_2$ 1157 cm^{-1}], [antisymmetric; $S^{16}O_e{}^{18}O_a$ 1401, $S^{17}O_e{}^{18}O_a$ 1389, $S^{18}O_2$ 1378 cm^{-1}]; *m/z* (NH_3 C.I.) 172 (35.2%), 173 (36.8%), 174 (28.5%); $C_4H_8SO_2{}^{17}O^{18}O \cdot NH_4^+$ requires *m/z* 173.

(4)-Methyl-2,2-[$^{18}O_2$]dioxo-1,3,2-dioxathiane. To a solution of ruthenium [$^{18}O_4$]tetraoxide (32 mg, 0.19 mmol) prepared as above was added a solution of *trans*-(4*R*)-methyl-2-[^{18}O]oxo-1,3,2-dioxathiane **1** (24 mg, 0.17 mmol) in chloroform (0.5 ml). Workup as above gave the title compound as a colorless oil (19 mg, 70%), R_f (ether) 0.35, ν_{max} [symmetric; $S^{16}O_e{}^{18}O_a$ 1172, $S^{18}O_2$ 1157 cm^{-1}], [antisymmetric; $S^{16}O_e{}^{18}O_a$ 1401, $S^{18}O_2$ 1378 cm^{-1}].

(1R)-3-Amino-1-methylpropyl [^{18}O]sulfate. (4*R*)-Methyl-(2*S*)-2,2-[^{18}O]dioxo-1,3,2-dioxathiane **5** (37 mg, 0.24 mmol) was dissolved in dry methanol (10 ml), and the solution cooled to 0°C and then saturated with gaseous ammonia. The solution was left stirring in the ice bath, allowed to warm to room temperature overnight, and then evaporated to afford the title compound as a white powdery material (41 mg, 100%), mp 165–170°C. $\delta_H(D_2O)$ 1.24 (3H, d, CH_3, $^3J_{HH}$ 7 Hz), 1.83 (2H, m, 2-*H*), 3.00 (2H, dt, 3-*H*, $^3J_{HH}$ 7 Hz, $^4J_{HH}$ 2 Hz), 4.44 (1H, m, 1-*H*).

(1R)-3-Hydroxy-1-methylpropyl [^{18}O]sulfate (pyridium salt). (1*R*)-3-Amino-1-methylpropyl [^{18}O]sulfate (41 mg, 0.24 mmol) was dissolved in water (0.45 ml) and acetic acid (0.15 ml). To the stirred solution at 0°C was added sodium nitrite (62 mg, 0.90 mmol). The mixture was stirred at 0°C for 7 h then evaporated to dryness. ^{1}H NMR analysis (60 MHz) showed the reaction to be incomplete; therefore the reaction was repeated by using a further addition of the reagents on the same scale. The mixture was allowed to warm to room temperature overnight and then evaporated to dryness.

The residue was flash chromatographed on silica (eluting with ethanol) to give a white semi-solid (67 mg). This was dissolved in methanol/water (v/v, 5 : 1) and

stirred with pyridinium Dowex to give a glassy material (81 mg), δ_H (D_2O) 1.25 (3H, d, CH_3, $^3J_{HH}$ 7 Hz), 1.72 (2H, q, 2-*H*, $^3J_{HH}$ 7 Hz), 3.57 (2H, t, 3-*H*, $^3J_{HH}$ 7 Hz), 4.49 (1H, sextet, 1-*H*, $^3J_{HH}$ 7 Hz), 8.00 and 8.55 (5H, m, C_5H_5N).

Cyclization of (1R)-3-hydroxy-1-methylpropyl [^{18}O]sulfate. The pyridinium salt of (1*R*)-3-hydroxy-1-methylpropyl [^{18}O]sulfate (0.20 mmol) was dissolved in dry acetonitrile (10 ml), the solution cooled to −35°C, and then sulfuryl chloride (55 μl, 0.68 mmol) added dropwise. The mixture was brought quickly to room temperature. TLC showed the presence of cyclic sulfate, R_f (ether) 0.35. The solution was evaporated and the residue partitioned between water (5 ml) and ether (3 × 5 ml). The combined ether extracts were dried ($MgSO_4$), filtered, and evaporated to leave a colorless liquid (11 mg). Flash chromatography (by elution with ether) gave an isotopomeric mixture of pure cyclic sulfate (10 mg, 27% overall yield from (4*R*)-methyl-(2*S*)-2,2-[^{18}O]dioxo-1,3,2-dioxathiane), δ_C ($CDCl_3$) (125 MHz) 20.75 (CH_3), 30.87 (*C*-5), 71.749 (^{18}O-*C*-6, 33% *C*-6), 71.784 (^{16}O-*C*-6, 67% *C*-6), 82.44 (*C*-4); ν_{max} [symmetric; $S^{16}O_2$ 1201, $S^{16}O_a{}^{18}O_e$ 1183, $S^{16}O_e{}^{18}O_a$ 1172 cm^{-1}], [antisymmetric; $S^{16}O_2$ 1414, $S^{16}O_e{}^{18}O_a$ 1401, $S^{16}O_a{}^{18}O_e$ 1392 cm^{-1}]; *m/z* (NH_3 C.I.) 170 (24.7%), 172 (75.3%); $C_4H_8SO_3{}^{18}O \cdot NH_4^+$ requires *m/z* 172.

(1R)-3-Amino-1-methylpropyl [(R)-^{16}O, ^{17}O, ^{18}O]sulfate ***11***. (4*R*)-Methyl-(2*R*)-2,2-[^{17}O, ^{18}O]dioxo-1,3,2-dioxathiane **9** (37 mg, 0.24 mmol) was dissolved in dry methanol (10 ml), and the solution cooled to 0°C and then saturated with gaseous ammonia. The solution was left stirring in the ice bath and allowed to warm to room temperature overnight. The solution was then evaporated to afford the title compound (41 mg, 100%), δ_H (D_2O) 1.24 (3H, d, CH_3, $^3J_{HH}$ 7 Hz), 1.83 (2H, m, 2-*H*), 3.00 (2H, dt, 3-*H*, $^3J_{HH}$ 7 Hz, $^4J_{HH}$ 2 Hz), 4.44 (1H, m, 1-*H*); mp 165–170°C.

(1R)-*3-Hydroxy-1-methylpropyl [(R)-^{16}O, ^{17}O, ^{18}O]sulfate (pyridinium salt)* ***13***. (1*R*)-3-Amino-1-methylpropyl [(*R*)-^{16}O, ^{17}O, ^{18}O]sulfate **11** (4 mg, 0.24 mmol) was dissolved in water (0.45 ml) and acetic acid (0.15 ml). To the stirred solution at 0°C was added sodium nitrite (62 mg, 0.90 mmol). The mixture was left stirring in the ice bath and allowed to warm slowly to room temperature overnight. The mixture was then evaporated to dryness.

The residue was flash chromatographed on silica eluting with ethanol to give an oil, R_f (methanol) 0.71. This material was converted to the pyridinium salt by dissolving the product in a mixture of methanol and water (v/v, 5 : 1) and stirring the solution with pyridinium Dowex for 15 min. The solution was filtered, the Dowex washed with methanol, and the combined filtrate and washings evaporated to leave an oil (44 mg, 73%), δ_H (D_2O) 1.25 (3H, d, CH_3, $^3J_{HH}$ 7 Hz), 1.72 (2H, q, 2-*H*, $^3J_{HH}$ 7 Hz), 3.57 (2H, t, 3-*H*, $^3J_{HH}$ 7 Hz), 4.49 (1H, sextet, 1-*H*, $^3J_{HH}$ 7 Hz), 8.00 and 8.55 (5H, m, C_5H_5N).

Cyclization of (1R)-3-hydroxy-1-methylpropyl [(R)-^{16}O, ^{17}O, ^{18}O]sulfate ***13***. The pyridinium salt of (1*R*)-3-hydroxy-1-methylpropyl [(*R*)-^{16}O, ^{17}O, ^{18}O]sulfate **13** (44 mg, 0.17 mmol) was dissolved in dry acetonitrile(10 ml), the solution cooled to −35°C, and then sulfuryl chloride (55 μl, 0.68 mmol) added dropwise. The mixture was brought quickly to room temperature. TLC showed the presence of cyclic sulfate, R_f (ether) 0.35. The solution was evaporated and the residue partitioned between water (5 ml) and ether (3 × 5 ml). The combined ether extracts were dried

($MgSO_4$), filtered, and evaporated to leave a colorless liquid (13 mg). This was flash chromatographed (eluting with ether) to give pure cyclic sulfate as an isotopomeric mixture (10 mg, 37%) (26% overall yield from (4*R*)-methyl-(2*R*)-2,2-[^{17}O, ^{18}O]dioxo-1,3,2-dioxathiane), δ_C ($CDCl_3$) (125 MHz) 20.73 (*C*H_3), 30.85 (*C*-5), 71.76 (^{18}O–*C*-6, 45% *C*-6), 71.80 (^{16}O–*C*-6, 55% *C*-6), 82.46 (*C*-4); ν_{max} [symmetric; $S^{16}O_2$ 1201, $S^{16}O_e{}^{17}O_a$ 1186, $S^{16}O_a{}^{18}O_e$ 1183, $S^{16}O_e{}^{18}O_a$ 1172, $S^{17}O_e{}^{18}O_a$ 1163, $S^{18}O_2$ 1157 cm^{-1}], [antisymmetric; $S^{16}O_2$ 1414, $S^{16}O_e{}^{17}O_a$, 1407, $S^{16}O_e{}^{18}O_a$ 1401, $S^{16}O_a{}^{18}O_e$ 1392, $S^{17}O_e{}^{18}O_a$ 1389, $S^{18}O_2$ 1378 cm^{-1}]; *m*/*z* (NH_3 C.I.) 172 (36.3%), 173 (36.5%), 174 (27.2%); $C_4H_8SO_2{}^{17}O{}^{18}O \cdot NH_4^+$ requires *m*/*z* 173.

(1R)-3-Amino-1-methylpropyl [(S)-[^{16}O, ^{17}O, ^{18}O]sulfate ***10.*** (4*R*)-Methyl-(2*S*)-2,2-[^{17}O, ^{18}O]dioxo-1,3,2-dioxathiane **8** (37 mg, 0.24 mmol) was dissolved in dry methanol (10 ml), and the solution cooled to 0°C and then saturated with gaseous ammonia. The solution was left stirring in the ice bath and allowed to warm to room temperature overnight. The solution was evaporated to afford the title compound (41 mg, 100%), δ_H (D_2O) 1.24 (3H, d, C*H*$_3$, $^3J_{HH}$ 7 Hz), 1.83 (2H, m, 2-*H*), 3.00 (2H, dt, 3-*H*, $^3J_{HH}$ 7 Hz, $^4J_{HH}$ 2 Hz), 4.44 (1H, m, 1-*H*); mp 165–170°C.

(1R)-3-Hydroxy-1-methylpropyl [(S)-^{16}O, ^{17}O, ^{18}O]sulfate (pyridinium salt) ***12.*** (1*R*)-3-Amino-1-methylpropyl [(*S*)-^{16}O, ^{17}O, ^{18}O]sulfate **10** (41 mg, 0.24 mmol) was dissolved in water (0.45 ml) and acetic acid (0.15 ml). The stirred solution was cooled to 0°C and then sodium nitrite (62 mg, 0.90 mmol) was added. The mixture was left stirring in the ice bath and allowed to warm slowly to room temperature overnight. The mixture was then evaporated to dryness.

The residue was flash chromatographed on silica eluting with ethanol to give an oily material, R_f (methanol) 0.71. This material was converted to the pyridinium salt by dissolving the product in a mixture of methanol and water (v/v, 5 : 1) and stirring the solution with pyridinium Dowex for 15 min. The solution was filtered, the Dowex washed with methanol, and the combined filtrate and washings evaporated to leave an oil (50 mg, 83%), δ_H (D_2O) 1.25 (3H, d, C*H*$_3$, $^3J_{HH}$ 7 Hz), 1.72 (2H, q, 2-*H*, $^3J_{HH}$ 7 Hz), 3.57 (2H, t, 3-*H*, $^3J_{HH}$ 7 Hz), 4.49 (1H, sextet, 1-*H*, $^3J_{HH}$ 7 Hz), 8.00 and 8.55 (5H, m, C_5H_5N).

*Cyclization of (1R)-3-hydroxy-1-methylpropyl [(S)-^{16}O, ^{17}O, ^{18}O]*sulfate ***12.*** The pyridinium salt of (1*R*)-3-hydroxy-1-methylpropyl [(*S*)-^{16}O, ^{17}O, ^{18}O]sulfate (50 mg, 0.23 mol) was dissolved in dry acetonitrile (10 ml), the solution cooled to −35°C, and sulfuryl chloride (55 μl, 0.68 mmol) added dropwise. The mixture was brought quickly to room temperature. TLC showed the presence of cyclic sulfate, R_f (ether) 0.35. The solution was evaporated and the residue partitioned between water (5 ml) and ether (3 × 5 ml). The combined ether extracts were dried ($MgSO_4$), filtered, and evaporated to leave a colorless liquid (17 mg). This was flash chromatographed (eluting with ether) to give pure cyclic sulfate as an isotopomeric mixture (13 mg, 42%) (35% overall yield from (4*R*)-methyl-(2*S*)-2,2-[^{17}O, ^{18}O] dioxo-1,3,2-dioxathiane); ν_{max} [symmetric; $S^{16}O_2$ 1201, $S^{16}O_a{}^{17}O_e$ 1192, $S^{16}O_a{}^{18}O_e$ 1183, $S^{16}O_e{}^{18}O_a$ 1172, $S^{17}O_a{}^{18}O_e$ 1170, $S^{18}O_2$ 1157 cm^{-1}], (antisymmetric; $S^{16}O_2$ 1414, $S^{16}O_e{}^{18}O_a$ 1401, $S^{16}O_a{}^{17}O_e$ 1401, $S^{16}O_a{}^{18}O_e$ 1392, $S^{17}O_a{}^{18}O_e$ 1384, $S^{18}O_2$ 1378 cm^{-1}]; *m*/*z* (NH_3 C.I.) 172 (35.8%), 173 (37.2%), 174 (27.0%); $C_4H_8SO_2{}^{17}O{}^{18}O \cdot NH_4^+$ requires *m*/*z* 173.

(1R)-3-[^{18}O]Hydroxy-1-methylpropyl sulfate. (1*R*)-3-Amino-1-methylpropyl sul-

fate (41 mg, 0.24 mmol) was dissolved in ^{18}O-water (0.15 ml) and acetic acid (0.15 ml). To the stirred solution at 0°C was added sodium nitrite (62 mg, 0.90 mmol). The mixture was left stirring in the ice bath, allowed to warm to room temperature overnight, and then evaporated to dryness.

The residue was flash chromatographed (eluting with methanol) to give an oil (15 mg, 36%), R_f(methanol) 0.73, δ_H (D_2O) 1.25 (3H, d, CH_3, $^3J_{HH}$ 7 Hz), 1.72 (2H, q, 2-*H*, $^3J_{HH}$ 7 Hz), 3.57 (2H, t, 3-*H*, $^3J_{HH}$ 7 Hz), 4.49 (1H, sextet, 1-*H*, $^3J_{HH}$ 7 Hz); δ_C(CD_3OD) (125 MHz) 21.55 (CH_3), 40.78 (*C*-2), 59.42(^{18}O–*C*-3, 60% *C*-3), 59.44 (^{16}O–*C*-3, 40% *C*-3), 74.41 (*C*-1); *m/z* (-FAB) 169 (50%), 171 (50%).

Cyclization of (1R)-3-[^{18}O]hydroxy-1-methylpropyl sulfate. (1*R*)-3-[^{18}O]Hydroxy-1-methylpropyl sulfate (15 mg, 87 μmol) was dissolved in dry acetonitrile (5 ml), the solution cooled to −35°C, and then sulfuryl chloride (10 μl, 0.12 mmol) added dropwise. The mixture was brought quickly to room temperature. TLC showed cyclic sulfate, R_f (ether) 0.35. The solution was evaporated and the residue rinsed with ether. The ethereal solution was evaporated and the residue flash chromatographed (eluting with ether) to give 4.5 mg (34%) of cyclic sulfate; ν_{max} [symmetric; $S^{16}O_2$ 1201 cm^{-1}], [antisymmetric; $S^{16}O_2$ 1414 cm^{-1}]; δ_C ($CDCl_3$) 20.75 (CH_3), 30.87 (*C*-2), 71.77 (*C*-3), 82.43 (*C*-1).

ACKNOWLEDGMENT

We gratefully acknowledge support for this work from the SERC.

REFERENCES

1. LOWE, G., AND SALAMONE, S. J. (1984) *J. Chem. Soc. Chem. Commun.*, 466.
2. PINCHAS, S., AND LAULICHT, I. (1971) Infrared Spectra of Labelled Compounds, Academic Press, London/New York.
3. POLO, S. R., AND WILSON, M. K. (1954) *J. Chem. Phys.* **22,** 900.
4. LOWE, G., AND SALAMONE, S. J. (1983) *J. Chem. Soc. Chem. Commun.*, 1392.
5. LICHTENBERGER, J., AND LICHTENBERGER, R. (1948) *Bull Soc. Chim. France*, 1002; Lichtenberger, J., and Durr, L. (1956) *Bull. Soc. Chim. France*, 664.
6. RISLEY, J. M., AND VAN ETTEN, R. L. (1979) *J. Amer. Chem. Soc.* **101,** 252; VEDERAS, J. C. (1980) *J. Amer. Chem. Soc.* **102,** 374; KING, J. E., SKONIECZNY, S., KHEMANI, K. C., AND STOTHERS, J. B. (1983) *J. Amer. Chem. Soc.* **105,** 6514; HANSEN, P. E. (1983) *Annu. Rep. NMR Spectrosc.* **15,** 105.
7. ANDERSON, R. C., AND SHAPIRO, M. J. (1984) *J. Org. Chem.* **49,** 1304.
8. MURAKAMI, S., HASADA, T., AND TAI, A. (1980) *Bull. Chem. Soc. Japan* **53,** 1356.