A Novel Mechanism of Enzymic Ester Hydrolysis

INVERSION OF CONFIGURATION AND CARBON-OXYGEN BOND CLEAVAGE BY SECONDARY ALKYL SULPHOHYDROLASES FROM DETERGENT-DEGRADING MICRO-ORGANISMS

By BARBARA BARTHOLOMEW, KENNETH S. DODGSON,*
GEORGE W. J. MATCHAM,† DUNCAN J. SHAW and GRAHAM F. WHITE
Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL,
Wales, U.K.

(Received 28 February 1977)

The hydrolysis was studied of potassium (+)-octan-2-yl sulphate by two analogous, optically stereospecific, secondary alkylsulphohydrolases purified from two detergent-degrading micro-organisms, *Comamonas terrigena* and *Pseudomonas* C12B. Polarimetry studies have shown that (+)-octan-2-yl sulphate prepared from (+)-octan-2-ol is hydrolysed by both enzymes to yield (–)-octan-2-ol. This inversion of configuration implies that the enzymes are catalysing the scission of the C–O bond of the C–O–S linkage, a type of bond scission apparently not hitherto encountered among hydrolytic enzymes acting on ester bonds. Enzymic hydrolysis of potassium (+)-octan-2-yl sulphate in the presence of $H_2^{18}O$ and analysis of hydrolysis products for the presence of ^{18}O has confirmed that C–O bond scission (and not O–S bond scission) occurs with both enzymes.

From the point of view of mechanistic organic chemistry the hydrolysis of sulphate esters is of considerable interest, because the position of cleavage of the C–O–S linkage depends both on the chemical nature of the sulphate ester and on the conditions under which hydrolysis occurs. Aryl sulphates appear generally to undergo exclusive O–S bond cleavage in acidic and neutral media (Spencer, 1958; Kice & Anderson, 1966; Benkovic, 1966), and predominantly O–S cleavage under alkaline conditions (Spencer, 1958; Benkovic & Lund, 1966; Fendler & Fendler, 1968). This is in keeping with the low tendency of aryl esters to undergo substitution reactions at the aryl carbon atom (Burkhardt *et al.*, 1936; Oae & Kiritani, 1965; March, 1968). The first step in the hydrolysis of five-membered cyclic sulphate diesters involves O–S cleavage in alkali, but in acid solution C–O cleavage is the rule. The product monoesters exhibit the opposite behaviour, with C–O cleavage in alkali and O–S cleavage in acid (Garner & Lucas, 1950; Brimacombe *et al.*, 1960; Kaiser *et al.*, 1963). Several simple alkyl sulphates also exhibit C–O bond fission in alkali and O–S bond fission in acid (Burwell, 1952; Kursanov & Kudryartsev, 1956; Kaiser *et al.*, 1963; Batts, 1966). In the particular case of butan-2-yl sulphate, where the sulphate group is attached to an asymmetric carbon atom, Burwell (1952) has shown that acid

hydrolysis leads to retention of configuration, although some racemization occurs. In alkali, however, C–O bond cleavage occurs and is accompanied by complete inversion of configuration at C-2.

Studies on the enzymic hydrolysis of aryl sulphates by a number of different arylsulphohydrolases (Spencer, 1958, 1959) have established that these enzymes operate by fission of the O–S bond, and it has since been tacitly assumed that this was a generalization that applied to all sulphohydrolase enzymes (Dodgson & Rose, 1975). However, the recent discovery that secondary alkylsulphohydrolase enzymes present in certain detergent-degrading micro-organisms exhibit optical stereospecificity (Matcham *et al.*, 1977), suggested that further studies should be undertaken before this generalization should be accepted. Two of these enzymes have recently been purified to homogeneity in our laboratories (K. S. Dodgson, G. W. J. Matcham & B. Bartholomew, unpublished work); one from cells of *Pseudomonas* C12B (the 'S1 enzyme' of Dodgson *et al.*, 1974) and the other from cells of a strain of *Comamonas terrigena* (the 'CS2 enzyme' of Fitzgerald *et al.*, 1975). Although originating from two different organisms, these two enzymes are analogous and appear to be specific for (+)-2-sulphate esters of secondary alcohols containing at least six carbon atoms (Matcham *et al.*, 1977). Potassium (+)-octan-2-yl sulphate, prepared from (+)-octan-2-ol, is the standard substrate now used for their assay.

The present work establishes that neither enzyme obeys the generalization outlined above, and provides,

* To whom reprint requests should be addressed.

† Present address: Department of Biochemistry, School of Medicine, University of Miami, P.O. Box 520875, Miami, FL 33152, U.S.A.

for the first time, examples of sulphohydrolases operating by fission of the C–O bond of the C–O–S linkage. Moreover, a further novel finding for sulphohydrolases, the fission of the C–O bond is accompanied by complete bond inversion.

Materials and Methods

Materials

^{18}O -enriched water (61.8 atom % excess of ^{18}O) was purchased from British Oxygen Co. (Prochem), London S.W.19, U.K., and racemic octan-2-ol from Aldrich Chemical Co., Milwaukee, WI, U.S.A. All other chemicals were the purest available from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K., or BDH Chemicals, Poole, Dorset, U.K.

Enzyme preparations

The CS2 enzyme was purified by the method of Matcham (1976) from extracts of cells of *C. terrigena* grown to the stationary phase (see Fitzgerald *et al.*, 1975). The final preparation was homogeneous on the basis of polyacrylamide-gel electrophoresis and had a specific activity of 768 units/mg of protein (see under 'Enzyme substrate and assay' for definition of unit).

The S1 enzyme was purified from extracts of stationary-phase cells of *Pseudomonas* C12B by a procedure which involved the following stages: (a) removal of nucleic acid with streptomycin sulphate; (b) removal of unwanted protein precipitating at 55% saturation with $(\text{NH}_4)_2\text{SO}_4$; (c) concentration and dialysis of the supernatant followed by chromatography on DEAE-cellulose (DE-52) with a linear gradient of Tris/HCl buffer (0.01–0.5 M, pH 7.8); (d) fractionation on Sephadex G-100 in 0.01 M-Tris/HCl buffer, pH 7.8; and (e) rechromatography on DEAE-cellulose (B. Bartholomew & K. S. Dodgson, unpublished work). The final preparation gave a single protein band on polyacrylamide-gel electrophoresis and had a specific activity of 566 units/mg of protein. In some experiments, a less pure preparation (approx. 200 units/mg of protein), but containing no other sulphohydrolase activity, was used. This preparation gave an additional protein band on polyacrylamide gels.

Enzyme substrate and assay

Racemic octan-2-ol was resolved by the method of Kenyon (1922) as described by Vogel (1956), a method which involved the formation of octan-2-yl hydrogen phthalate and its resolution by fractional crystallization of the brucine salt. The (+)-octan-2-ol subsequently recovered from the resolved phthalate

ester gave a specific optical rotation $[\alpha]_{546}^{20} = +11.8^\circ$ (c 0.1 g/ml in ethanol). It was converted into the corresponding ester sulphate (potassium salt) by treatment with pyridine- SO_3 complex (made from SO_3 and not from chlorosulphonic acid) as described by Matcham (1976). The final product had $[\alpha]_{546}^{20} = +3.25^\circ$ (c 0.05 g/ml in water).

Activity of the CS2 enzyme was measured by determining the rate of release of inorganic sulphate in incubation mixtures containing 18 mM-potassium (+)-octan-2-yl sulphate, 100 mM-Tris/maleate buffer, pH 7.5, and suitably diluted enzyme. Incubation was at 30°C and liberated sulphate was determined by the BaCl_2 /gelatin method (method B) of Dodgson (1961) as modified by Thomas & Tudball (1967). A unit of enzyme activity is defined as that producing $1\text{ }\mu\text{mol}$ of SO_4^{2-} /min (cf. Dodgson *et al.*, 1974). The S1 enzyme was measured in the same way, except that the buffer used was 100 mM-Tris/HCl, pH 8.2, and the concentration of substrate was 7.5 mM.

Polarimetry

Optical rotations of solution were measured in a 1 cm path-length cell at either 546 nm (mercury lamp) or the sodium D-line, by using an NPL automatic polarimeter, type 243 (Thorn Automation, Nottingham, U.K.). Except where otherwise stated, the solvents used for (+)-octan-2-ol and its sulphate ester were ethanol and water respectively.

The following experiments were performed to determine the optical activity of the products of enzymic and acid- and base-catalysed hydrolysis of (+)-octan-2-yl sulphate.

CS2 enzyme. Potassium (+)-octan-2-yl sulphate (202 mg, 0.815 mmol) was incubated at 30°C with the pure enzyme (22 units) in a total volume of 25 ml of 0.4 M-Tris/maleate buffer, pH 7.5. After 2 h, a sample was removed and the liberated sulphate measured, to confirm that the reaction was complete. The liberated alcohol was then extracted with diethyl ether ($3 \times 10\text{ ml}$). The ether was removed by gentle evaporation in a stream of N_2 at 20°C and the residue dissolved in 5 ml of ethanol, for determination of its optical rotation.

S1 enzyme. Very small amounts only of the pure enzyme were available, so for this particular experiment the less pure preparation (200 units/mg) was used. The incubation mixture contained 37.2 mg (0.15 mmol) of potassium (+)-octan-2-yl sulphate and enzyme (3 units) in a total volume of 4 ml of 0.25 M-Tris/HCl buffer, pH 7.5. The reaction was allowed to proceed to approx. 95% completion (95 min) before the octan-2-ol was extracted from the mixture with diethyl ether ($3 \times 4\text{ ml}$) and its optical rotation in ethanolic solution (5 mg/ml) was determined as described above. The concentration of octan-2-ol was confirmed by g.l.c. analysis.

Acid and alkali. Hydrolysis mixtures were prepared as for the CS2 enzyme except that enzyme and buffer were omitted and 1M-HCl or 5M-NaOH was present in the final solutions. Solutions were maintained at 100°C under reflux. Hydrolysis with acid was complete within 10h, whereas only 60% hydrolysis was achieved in 42h with alkali. Liberated alcohol was extracted as above and specific optical rotations were determined, with allowance for incomplete hydrolysis where appropriate.

¹⁸O incorporation

Experiments were conducted to test for ¹⁸O incorporation from solvent into one or other of the hydrolysis products (octan-2-ol or SO₄²⁻ ions) in the presence of CS2 or S1 enzymes. Incubation mixtures contained 15 μmol of potassium (+)-octan-2-yl sulphate, 0.1 ml of 1M-Tris/HCl buffer, pH 7.5, 0.2 ml of H₂¹⁸O, pure enzyme and water to a final volume of 0.4 ml. With the CS2 enzyme approx. 3.48 enzyme units were used over an incubation period of 50 min at 30°C. With S1 enzyme, 0.12 unit was used over an incubation period of 18 h. Preliminary experiments showed that these conditions resulted in greater than 95% hydrolysis of the substrate, and in both cases normal hydrolysis curves were obtained. The octan-2-ol and SO₄²⁻ ions liberated by the enzyme were then separated and analysed for ¹⁸O content as follows.

The liberated alcohol was extracted with diethyl ether (2 × 0.4 ml) and the extract was then analysed for ¹⁸O labelling by mass spectrometry in a Finnegan 4000 GCMS instrument (Finnegan Instruments, Hemel Hempstead, Herts., U.K.). With unlabelled octan-2-ol, ionization by electron impact produced a spectrum showing no molecular ion (mol.wt. = 130). The largest fragment detected had a mass/charge ratio (*m/e*) of 115, corresponding to loss of a methyl group. Since this ion still contains oxygen it was used to estimate the ¹⁶O/¹⁸O ratio in the samples. The selected ion-monitoring technique was used to determine the relative amounts of ions of mass 115 (from octan-2-[¹⁶O]ol) and 117 (from octan-2-[¹⁸O]ol) in each sample. Four runs were performed for each sample and average values of the atom % of ¹⁸O present were calculated from the peak heights.

The SO₄²⁻ ions remaining in the aqueous phase after removal of octan-2-ol were precipitated as BaSO₄ and analysed for ¹⁸O content by a modification (Tudball & Thomas, 1972) of the i.r. method of Spencer (1959). The ¹⁸O content in BaSO₄ was determined by comparing the peaks at 961 cm⁻¹ (which appears when BaS¹⁸O¹⁶O₃ is present) and 981 cm⁻¹.

Control hydrolyses were performed for both CS2 and S1 enzymes in the absence of any H₂¹⁸O to determine the natural abundance of ¹⁸O in the octan-2-ol and SO₄²⁻ ions.

In view of the long incubation period necessary in the experiments with the S1 enzyme, it was necessary to determine whether any exchange occurred between H₂¹⁸O and octan-2-ol or SO₄²⁻ ions in the presence of the enzyme. The potassium octan-2-yl sulphate was therefore replaced by an equimolar mixture of octan-2-ol and Na₂SO₄. After incubation in the presence of S1 enzyme for 18 h, less than 0.4% of each product had undergone exchange with H₂¹⁸O.

Results and Discussion

Polarimetry experiments

The results in Table 1 show that both CS2 and S1 enzymes act on potassium (+)-octan-2-yl sulphate with the liberation of (–)-octan-2-ol ([α]_D²⁰ = –11.8°). Clearly the optical stereospecificity that these enzymes exhibit for their substrate is extended to include a similar stereospecificity in their catalytic action in releasing an optically active alcohol.

To interpret these results in terms of a molecular mechanism of hydrolysis, a knowledge of the configurational relationship between (+)-octan-2-yl sulphate and (–)-octan-2-ol becomes essential. The following available evidence indicates that (+)-octan-2-yl sulphate and (+)-octan-2-ol are configuratively equivalent.

(a) The slow alkaline hydrolysis, involving inversion of configuration, and the rapid acid hydrolysis, with initial retention of configuration followed by some racemization, are well established for secondary alkyl sulphates (Burwell, 1952; Batts, 1966). The results in Table 1, which show that (+)-octan-2-yl sulphate produces predominantly (+)-octan-2-ol in acid and (–)-octan-2-ol in alkali, are consistent with this pattern only if (+)-octan-2-yl sulphate and (+)-octan-2-ol are configuratively related.

(b) For the present experiments (+)-octan-2-yl sulphate was prepared by sulphation of (+)-octan-2-ol by pyridine-SO₃ complex, and it is difficult to envisage a mechanism for this reaction which involves simultaneous inversion and sulphation of the alcohol. Indeed, sulphation of butan-2-ol with this

Table 1. *Specific optical rotations* [α]_D²⁰ *of octan-2-ol produced by enzymic, acid- and base-catalysed hydrolysis of potassium (+)-octan-2-yl sulphate*

Conditions used for the hydrolyses are described in the text. Rotations were measured in ethanol at 546 nm.

Enzyme or catalyst	[α] _D ²⁰ of liberated alcohol
Enzyme CS2	–10.4°
Enzyme S1	–10.6°
1M-HCl	+1.0°
5M-NaOH	–2.9°

reagent is already known to proceed with complete retention of configuration (Burwell, 1949).

(c) It has been established by chemical synthesis (Doering & Young, 1952) and enzyme-specificity studies (Dickinson & Dalziel, 1967) that (+)-butan-2-ol and (+)-octan-2-ol have the same configuration. According to the Rule of Shift (Freudenberg, 1933), if two similarly constituted asymmetric molecules are chemically altered in the same way, then the change in rotation will be in the same direction in each case (and usually of the same order of magnitude). The essential requirement (Mills & Klyne, 1954), that of similarity of structure, is satisfied for the (+)-butan-2-ol and (+)-octan-2-ol pair, and hence sulphation of the hydroxyl group by pyridine-SO₃ in each case may be expected to produce similar changes in rotation. For (+)-butan-2-ol ($[\alpha]_D^{20} = +10.97^\circ$), sulphation produces the configuratively equivalent (+)-butan-2-yl sulphate ($[\beta]_D^{20} = +10.7^\circ$; Burwell, 1952). Evidently sulphation here produces a very small negative change in rotation. Thus pyridine-SO₃ sulphation of (+)-octan-2-ol ($[\alpha]_{546}^{20} = +11.8^\circ$) may be expected to produce (+)-octan-2-yl sulphate with retention of configuration and a smaller but still positive specific optical rotation. This is consistent with a direct configurational relationship between (+)-octan-2-ol ($[\alpha]_{546}^{20} = +11.8^\circ$) and (+)-octan-2-yl sulphate ($[\alpha]_{546}^{20} = +3.25^\circ$).

(d) Mayers *et al.* (1969) reported the preparation of (+)-octan-2-yl sulphate from (–)-octan-2-ol using chlorosulphonic acid in dioxan. After the liberation of (–)-octan-2-ol by solvolysis of the preparation in dioxan, these authors propose complete retention of configuration throughout, thus apparently relating (+)-octan-2-yl sulphate and (–)-octan-2-ol. At first sight, this is in direct contrast with previous evidence. However, Mayers *et al.* (1969) used ethanol/water (4:1, v/v) as solvent for octan-2-yl sulphate and the sodium D-line for polarimetry, whereas water and

the mercury green line at 546 nm have been used in the present study. It is possible that the change in solvent and/or the change in wavelength for polarimetric measurements would produce different signs of rotation for the same octan-2-yl sulphate isomer. This is the case, since we find that although changing the wavelength has a small effect on the rotation of octan-2-ol and its sulphate, changing the solvent produces a change in sign of rotation for octan-2-yl sulphate (Table 2). Thus pyridine-SO₃ sulphation of (+)-octan-2-ol ($[\alpha]_{546}^{20} = +11.8^\circ$, *c* 0.1 g/ml in ethanol) produces octan-2-yl sulphate with $[\alpha]_{546}^{20} = +3.25^\circ$ in water, but -0.79° in ethanol/water (4:1, v/v). A similar effect was observed for the sulphate prepared from (–)-octan-2-ol. The results shown in Table 2 make the report of Mayers *et al.* (1969) entirely consistent with our contention that (+)-octan-2-ol (measured in ethanol) and (+)-octan-2-yl sulphate (measured in water) are configuratively equivalent. It seems certain then that (+)-octan-2-ol and (+)-octan-2-yl sulphate have the same configuration. Since (+)-octan-2-ol is also related configuratively to (+)-butan-2-ol (Doering & Young, 1952), which in turn is related to D(+)-glyceraldehyde (Mills & Klyne, 1954), the optical isomers of octan-2-ol and octan-2-yl sulphate should now be referred to as D(+)-octan-2-ol and D(+)-octan-2-yl sulphate.

Experiments with ¹⁸O

Since D(+)-octan-2-yl sulphate and D(+)-octan-2-ol are configuratively related, it follows that the hydrolyses of D(+)-octan-2-yl sulphate catalysed by enzymes CS2 and S1 to give L(–)-octan-2-ol and SO₄^{2–} ions must involve Walden inversion of configuration at the asymmetric carbon atom. This in turn implies that the C–O bond of the ester linkage is broken during the hydrolysis. As a means of checking this important implication, experiments were conducted in which the enzymic hydrolyses were

Table 2. *Specific optical rotations of (+)- and (–)-octan-2-ols and the corresponding sulphate esters under various conditions*
(a) Present results; the results in (b) are those of Mayers *et al.* (1969), where the sulphate ester was prepared by sulphation with chlorosulphonic acid in dioxan. In the present work pyridine-SO₃ reagent was used as sulphating agent. Ethanol/water mixtures were 4:1 (v/v).

Substance	Concn. (g/ml)	Solvent	Specific optical rotation	
			546 nm	Sodium D-line
(a) (+)-Octan-2-ol	0.1	Ethanol	+11.8°	+10.5°
Octan-2-yl sulphate	0.05	Water	+3.25°	+2.5°
		Ethanol/water	–0.79°	–0.6°
(–)-Octan-2-ol	0.1	Ethanol	–11.8°	–10.8°
Octan-2-yl sulphate	0.05	Water	–3.25°	–2.1°
		Ethanol/water	+0.83°	+0.5°
(b) (–)-Octan-2-ol	0.086	Ethanol	—	–10.05°
Octan-2-yl sulphate	0.073	Ethanol/water	—	+1.0°

Table 3. Incorporation of ^{18}O during the enzymic hydrolysis of (+)-octan-2-yl sulphate in the presence of H_2^{18}O . Conditions used for the hydrolyses are described in the text. The atom % excess of the H_2^{18}O was 30.9.

	Atom % excess					
	Calc. for C-O split		Calc. for O-S split		Found	
	Octan-2-ol	SO_4^{2-}	Octan-2-ol	SO_4^{2-}	Octan-2-ol	SO_4^{2-}
Alkylsulphohydrolase						
Enzyme CS2	30.9	0	0	7.7	31.6	0
Enzyme S1	30.9	0	0	7.7	28.2	0

allowed to proceed in the presence of H_2^{18}O . Cleavage of the C-O bond would result in the production of octan-2- ^{18}O ol, whereas O-S cleavage would produce $\text{S}^{18}\text{O}^{16}\text{O}_3^{2-}$ ions. The results (Table 3) confirm that C-O bond fission does indeed occur for both enzymes, with an associated inversion of configuration at the asymmetric carbon atom.

Several important considerations now arise as a result of these findings. Firstly, the question arises as to whether the other alkylsulphohydrolases produced by *C. terrigena* (see Fitzgerald *et al.*, 1975) and *Pseudomonas* C12B (see Dodgson *et al.*, 1974) also attack the C-O bond. In *C. terrigena* there is preliminary evidence that a partially purified preparation of the CS1 enzyme releases (+)-decan-2-ol from racemic decan-2-yl sulphate. Since CS1 enzyme is specific for D(-)-octan-2-yl sulphate [and therefore probably (-)-decan-2-yl sulphate], this enzyme also probably inverts the configuration during hydrolysis. With the two primary alkylsulphohydrolases (designated P1 and P2) of *Pseudomonas* C12B, which are active towards primary alkyl sulphates such as sodium dodecyl sulphate, clearly inversion of configuration does not arise. However, it will certainly be interesting to determine the position of bond scission in these cases.

Secondly, as the hydrolysis of optically active alkyl 2-sulphates produces optically active alcohols, the question of optical specificity extends to the subsequent metabolism of the liberated alcohols. One possibility is that there is a stereospecific alcohol dehydrogenase for each isomer. However, since oxidation leads to loss of optical activity, a more logical arrangement would be either a non-stereospecific dehydrogenase or an alcohol racemase channelling both enantiomers through a single alcohol dehydrogenase. With regard to the first of these two suggestions, it is noteworthy that horse liver dehydrogenase is active towards a number of secondary alcohols of various chain lengths, and moreover it is active towards both positional and optical isomers. In contrast, the yeast enzyme will oxidize only (+)-butan-2-ol and (+)-octan-2-ol (Dickinson & Dalziel, 1967). Certainly a study of the alcohol dehydrogenases of these micro-organisms

may relate directly to the stereospecificity of the secondary alkylsulphohydrolases and throw more light on the metabolic capabilities of the organisms.

Thirdly, C-O bond cleavage by the CS2 and S1 alkylsulphohydrolases is in direct contrast with the general pattern for the sulphohydrolases so far examined, all of which attack the O-S bond (Spencer, 1958, 1959; Sampson *et al.*, 1975; Dodgson & Rose, 1975). However, all previous studies have been made with Type-I and Type-II arylsulphohydrolases, and aryl substrates generally tend to resist rupture of the aryl C-O bond under a wide variety of conditions. Thus it may be that similar studies on other types of sulphohydrolase would reveal as rich a pattern of mechanisms operating in the enzymic hydrolysis of sulphate esters as exists for the non-enzymic hydrolysis.

Finally, on a broader front, the Enzyme Nomenclature Section of the IUPAC-IUB Commission on Biochemical Nomenclature (see Florkin & Stotz, 1973) recognizes seven different classes of hydrolytic enzyme acting on ester bonds, six of which are of the type acid-O-alcohol (the exception being thioesters). A review of the relevant literature indicates that the only mode of action yet observed for these enzymes is cleavage of the acid-oxygen bond rather than the oxygen-alcohol bond. The list includes carboxylic ester hydrolases (EC 3.1.1), e.g. carboxyl esterases (Krisch, 1971), fatty acid esterases (Hofstee, 1960) and acetylcholinesterase (Rosenberry, 1975); phosphoric monoester hydrolases (EC 3.1.3), e.g. alkaline phosphatase (Stein & Koshland, 1952), acid phosphatase (Cohn, 1959), 5'-nucleotidase (Koshland & Springhorn, 1956), glucose 6-phosphatase (Nordlie, 1971) and fructose 1,6-bisphosphatase (Pontremoli *et al.*, 1965); phosphoric diester hydrolases (EC 3.1.4), e.g. spleen nuclease and phosphodiesterase, pancreatic ribonuclease (Hilmoe *et al.*, 1961), and phospholipase D (Heller *et al.*, 1975); sulphuric ester hydrolases (EC 3.1.6), e.g. arylsulphohydrolases from several sources (Spencer, 1958; Sampson *et al.*, 1975). Consequently, the discovery in the present work of sulphuric ester hydrolases which attack the alcohol-oxygen bond adds a new dimension to the field of ester hydrolase enzymes in general.

We thank Professor R. Gillard and Dr. D. Games of the Department of Chemistry for providing polarimetry facilities and performing mass spectrometry respectively, and Professor W. J. Payne and Dr. J. W. Fitzgerald of the Department of Microbiology, University of Georgia, for their invaluable help with the large-scale fermentation of the micro-organisms. G. W. J. M. acknowledges receipt of a Science Research Council Enzyme Technology Studentship. The work was supported by a Science Research Council project grant and a NATO travel grant to K. S. D.

References

- Batts, B. D. (1966) *J. Chem. Soc. B* 551–555
 Benkovic, S. J. (1966) *J. Am. Chem. Soc.* **88**, 5511–5515
 Benkovic, S. J. & Lund, P. (1966) *J. Am. Chem. Soc.* **88**, 5504–5511
 Brimacombe, J. S., Foster, A. B., Hancock, E. B., Overend, W. G. & Stacey, M. (1960) *J. Chem. Soc.* 201–211
 Burkhardt, G. N., Ford, W. G. K. & Singleton, E. (1936) *J. Chem. Soc.* 17–25
 Burwell, R. L. (1949) *J. Am. Chem. Soc.* **71**, 1769–1771
 Burwell, R. L. (1952) *J. Am. Chem. Soc.* **74**, 1462–1466
 Cohn, M. (1959) *J. Cell. Comp. Physiol.* **54**, Suppl. 1, 17–31
 Dickinson, F. M. & Dalziel, K. (1967) *Biochem. J.* **104**, 165–172
 Dodgson, K. S. (1961) *Biochem. J.* **78**, 312–319
 Dodgson, K. S. & Rose, F. A. (1975) in *Metabolic Pathways* (Greenberg, D. M., ed.), 3rd edn., vol. 7, pp. 359–431, Academic Press, New York and London
 Dodgson, K. S., Fitzgerald, J. W. & Payne, W. J. (1974) *Biochem. J.* **138**, 53–62
 Doering, W. von E. & Young, R. W. (1952) *J. Am. Chem. Soc.* **74**, 2997–2999
 Fendler, E. J. & Fendler, J. H. (1968) *J. Org. Chem.* **33**, 3852–3859
 Fitzgerald, J. W., Dodgson, K. S. & Matcham, G. W. J. (1975) *Biochem. J.* **149**, 477–480
 Florin, M. & Stotz, E. H. (eds.) (1973) *Comprehensive Biochemistry*, 3rd edn., vol. 13, p. 20, Elsevier, Amsterdam, London and New York
 Freudenberg, K. (1933) *Ber. Dtsch. Chem. Ges.* **66**, 177–194
 Garner, H. K. & Lucas, H. J. (1950) *J. Am. Chem. Soc.* **72**, 5497–5501
 Heller, M., Mozes, N. & Maes, E. (1975) *Methods Enzymol.* **35**, 226–232
 Hilmoe, R. J., Heppel, L. A., Springhorn, S. S. & Koshland, D. E. (1961) *Biochim. Biophys. Acta* **53**, 214–215
 Hofstee, B. H. J. (1960) *Enzymes 2nd Ed.* **4**, 485–500
 Kaiser, E. T., Panar, M. & Westheimer, F. H. (1963) *J. Am. Chem. Soc.* **85**, 602–607
 Kenyon, J. (1922) *J. Chem. Soc. Trans.* 2540–2542
 Kice, J. L. & Anderson, J. M. (1966) *J. Am. Chem. Soc.* **88**, 5242–5245
 Koshland, D. E. & Springhorn, S. S. (1956) *J. Biol. Chem.* **221**, 469–476
 Krisch, K. (1971) *Enzymes 3rd Ed.* **5**, 43–69
 Kursanov, D. N. & Kudryartsev, R. V. (1956) *J. Gen. Chem. U.S.S.R. (Engl. Transl.)* **26**, 3323–3325
 March, J. (1968) *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, p. 373, McGraw-Hill, New York
 Matcham, G. W. J. (1976) Ph.D. Thesis, University of Wales
 Matcham, G. W. J., Bartholomew, B., Dodgson, K. S., Fitzgerald, J. W. & Payne, W. J. (1977) *FEMS Lett.* **1**, 197–199
 Mayers, G. L., Ponsada, M. & Haines, T. H. (1969) *Biochemistry* **8**, 2981–2986
 Mills, J. A. & Klyne, W. (1954) *Prog. Stereochem.* **1**, 177–222
 Nordlie, R. C. (1971) *Enzymes 3rd Ed.* **4**, 543–610
 Oae, S. & Kiritani, R. (1965) *Kagaku To Kogyo (Tokyo) (Bull. Chem. Soc. Jpn.)* **38**, 765–770
 Pontremoli, S., Traviello, S., Luppis, B. & Wood, W. A. (1965) *J. Biol. Chem.* **240**, 3459–3463
 Rosenberry, T. L. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol.* **43**, 103–218
 Sampson, E. J., Vergara, E. V., Feder, J. M., Funk, M. O. & Benkovic, S. J. (1975) *Arch. Biochem. Biophys.* **169**, 372–383
 Spencer, B. (1958) *Biochem. J.* **69**, 155–159
 Spencer, B. (1959) *Biochem. J.* **73**, 442–447
 Stein, S. S. & Koshland, D. E. (1952) *Arch. Biochem. Biophys.* **39**, 229–230
 Thomas, J. H. & Tudball, N. (1967) *Biochem. J.* **105**, 467–472
 Tudball, N. & Thomas, P. (1972) *Biochem. J.* **126**, 187–191
 Vogel, A. I. (1956) *A Textbook of Practical Organic Chemistry*, pp. 505–507, Longmans, London