Gerard J. Mulder

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Sulfation of Drugs and Related Compounds

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His current research interests include the role of sulfation and glucuronidation in the detoxification and toxification of xenobiotics with special attention to the availability of the various co-substrates for conjugation.

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Chapter 1

INTRODUCTION

G. J. Mulder

Sulfate conjugation was discovered around 1875 by Baumann.[1-3] He isolated phenyl sulfate from the urine of a patient who had been treated with phenol; later he and his group demonstrated the presence of several sulfated phenols in urine, and studied some properties of sulfation.[4,5] These sulfate esters constituted the so-called "ethereal sulfate" fraction; they were considered to be "ethers" of the organic aryl group and the $-SO_3$ group: Ar–O–SO_3H. In fact, these compounds are half-esters of sulfuric acid and name "ethereal sulfate" is only used these days for historical reasons.

The chemical mechanism of the sulfate conjugation remained obscure at that time; it was believed that inorganic sulfate reacted directly with the substrate by enzymatic catalysis. However, between 1944 and 1956 the role of adenosine 3′-phosphate 5′-sulfatophosphate (PAPS), the cosubstrate of sulfation, was discovered by Lipmann's group and others. Only when the chemical structure of this cosubstrate had been elucidated, a clear picture of the mechanism of sulfation emerged.

In the meantime, R.T. Williams had started his work on the metabolism in vivo of foreign compounds (xenobiotics), of which many were excreted in bile or urine as sulfate conjugates, often after phase I metabolism (oxidation, reduction, or hydrolysis). The fate of numerous compounds in vivo was investigated by his group. Also, the mechanisms of their excretion in bile and urine were studied; in general, sulfate conjugates were more rapidly eliminated than the parent compounds, and usually were much less pharmacologically active. However, this was not always the case; several aromatic amines, for instance, become extremely reactive and potentially toxic upon sulfation.

Sulfate activation and conjugation received relatively little further interest once the main characteristics of PAPS synthesis and its role in sulfate transfer had been discovered. Probably this was due to the idea current at that time, that sulfation was a purely detoxifying reaction. Moreover, for sulfation of endogenous compounds, an ample supply of sulfate seemed to be available and, in the case of high doses of xenobiotics, usually glucuronidation with its much higher capacity could easily take over when sulfation capacity was insufficient, or inorganic sulfate depleted. The purification to homogeneity of the sulfotransferases had to wait until the late 1970s. This lack in interest in sulfation is further illustrated by the surprising fact that the second report on purification of APS kinase, one of the enzymes of sulfate activation, appeared only in 1973, 15 years after the first report in 1958; since then little has been reported on this enzyme. ATP-sulfurylase, the enzyme that catalyzes the first step of sulfate activation, has been extensively characterized, mainly, it seems, because its product APS is important in many bacterial strains as an electron acceptor. The second activation step is usually not necessary, since sulfate reduction in most of these microorganisms seems to require APS rather than PAPS.

The overall process of sulfation is shown in Figure 1. The supply of inorganic sulfate required for sulfation of both endogenous acceptor groups and xenobiotics comes mainly from the food under normal, nonfasting conditions; both absorption of inorganic sulfate and sulfoxidation of cysteine deliver inorganic sulfate to cells and tissues (Chapter 3). Under fasting conditions, inorganic sulfate can be supplied by catabolism of proteins and other macromolecules, especially the highly sulfated glycosaminoglycans.

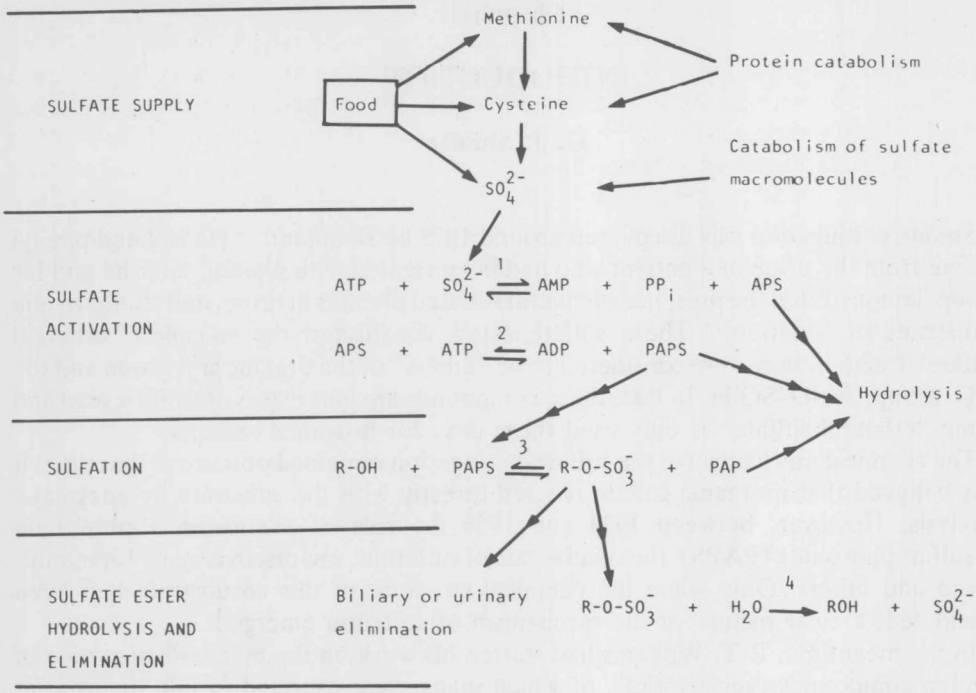

FIGURE 1. Sulfate metabolism and sulfation: 1. ATP-sulfurylase; 2. APS kinase; 3. Sulfotransferases; 4. Sulfatases.

Activation of inorganic sulfate to the group-donating cosubstrate, PAPS, takes place in a two-step reaction, catalyzed by ATP-sulfurylase and APS kinase. In higher animals PAPS seems to be used exclusively for sulfation, but in many microorganisms both APS and PAPS are electron acceptors; PAPS is required for the synthesis of cysteine. In most, if not all tissues of mammals, the sulfate activating system seems to be present (Chapter 4).

Sulfation of drugs and endogenous compounds occurs in most, probably all, animals and plants. A group of sulfotransferases catalyzes this transfer to various substrates; the specificity of these enzymes is derived from endogenous substrates, or xenobiotics from the natural environment. Purification and characterization of these sulfotransferases, as far as they use low-molecular weight substrates, is discussed in Chapter 5.

Under physiological conditions both sulfate activation and sulfate transfer take place in the same cell. When sulfation of a compound is studied in vivo therefore, the outcome is the result of complicated interactions in the cell. In order to study the factors that affect sulfation rate in vivo, in recent years isolated perfused organs and especially isolated cell preparations (mainly hepatocytes) have been used to elucidate the factors that determine the overall sulfation process in vivo. Availability of sulfate, substrate specificity and kinetics of the transferases, competition between glucuronidation and sulfation for the same substrate, and pharmacokinetic factors clearly all play a role. Selective inhibition of sulfation is an important tool to study the role of sulfation in detoxification and toxification (Chapter 6).

Once the sulfate conjugates are synthesized, they will be exposed to all the mechanisms that the body possesses to eliminate them. One of the main reasons why they are sulfated in the first place is that they could not be eliminated rapidly enough from the body. Thereby, sulfation gets a chance to convert them to the sulfate

conjugates that are usually more rapidly excreted. However, in several cases, even the sulfate conjugate cannot be eliminated rapidly enough, and it may be hydrolyzed again, yielding the original compound. Alternatively, the sulfate conjugate may be modified by other enzymes (e.g., by oxidation and reduction) as occurs with several steroid sulfates. Final elimination from the body takes place in urine and bile (Chapter 7).

Usually, sulfation means detoxification; however, steroid sulfates may be a storage form for some steroids that can be kept as a "silent reserve," or be modified specifically (Chapters 6 and 7). In some cases the sulfate conjugates are very unstable, chemically extremely reactive compounds that may be involved as ultimate carcinogens in carcinogenesis of the parent compound (Chapter 8). Whether sulfate conjugation means detoxification or toxification therefore depends on the substrate used.

In this book the sulfation of macromolecules will not be covered because with these substrates very different factors play a role (e.g., the macromolecular nature of the substrate), and different physiological processes are involved. For similar reasons the sulfation of lipids is not covered. In the various chapters, references will be given where possible, to recent reviews on these subjects.

The only previous book about sulfation, by Roy and Trudinger,[6] appeared in 1970, and dealt with all aspects of sulfation. This book gives an excellent review of the literature up until 1969; since then many new data have become available. A more complete survey of all types of sulfur-containing biological compounds is given in a recent volume in the series *Metabolic Pathways*.[7] A recent Ciba symposium covers the same, wide field.[8] With the increasing interest in sulfation in respect to toxification and detoxification of xenobiotics in mind, we have concentrated on the aspects that are most intimately connected with drug metabolism.

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Chapter 2

THE CHEMISTRY OF SULFATE ESTERS AND RELATED COMPOUNDS

A. B. Roy

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I. INTRODUCTION

In biochemistry, the name sulfate ester is given to a mono ester of sulfuric acid and a hydroxyl-containing compound which is usually prepared as an alkali metal salt, $R.OSO_3^-M^+$. In solution these are fully ionized so that the nature of the cation may not be important, but this is by no means always the case, either in biochemical or chemical reactions, and instead of simply writing, for example, phenyl sulfate, one should write potassium phenyl sulfate, barium phenyl sulfate, etc., as the case may be. In a more chemical context this avoids confusion with the corresponding diesters, which do not occur naturally. For example, diphenyl sulfate, $C_6H_5.OSO_2O.C_6H_5$, may also incorrectly be called phenyl sulfate. In what follows, terms such as phenyl sulfate will be used to mean the mono ester, and the associated cation will only be mentioned where it is important or where confusion could occur.

Chemical Abstracts indexes these compounds in two ways: the esters of simple compounds are listed under "Sulfuric acid, esters: mono X ester Y salt" while those of more complex alcohols or phenols are listed under the parent compounds.

Some other types of compounds are fairly closely related to the sulfate esters; these are the thiosulfate esters or Bunte salts, $R.SSO_3^-M^+$; the sulfamates, $R.NHSO_3^-M^+$; and the sulfatophosphates which contain the grouping $-O.PO(OH).OSO_3^-$. All of these, like the sulfate esters themselves, yield SO_4^{2-} with varying ease on acid hydrolysis. The first two groups of compounds are presently listed in *Chemical Abstracts* under the headings thiosulfuric acid, mono X ester Y salt, and sulfamic acid, mono X ester Y salt, respectively. The biologically important sulfatophosphates are listed under the parent nucleotide, for example, adenylic acid, monoanhydride with sulfuric acid.

Representatives of all the above types of compounds are found in biological systems as can be seen from Table 1 which lists those substances whose chemistry will be considered in this chapter. Further details of their chemistry can be found in the appropriate chapters of References 1 to 4.

II. SULFATE ESTERS

As has already been pointed out, these are the mono esters of sulfuric acid and a hydroxyl-containing compound which may be aliphatic, alicyclic, aromatic, or heterocyclic and which may be a *C*-hydroxy or an *N*-hydroxy compound. Obviously, the properties of these esters will depend very much upon the structure of the organic part of the molecule, and here it will be possible to give no more than general indications of their chemistry.

A. Synthesis of Sulfate Esters

There are two general methods which have been used for the preparation of many different sulfate esters and a host of minor methods which have been used less frequently, often only for the preparation of specific compounds. Many of the methods can be adapted for the preparation of [^{35}S]-sulfate esters but, because of the commercial availability of the reagents, those using $H_2{}^{35}SO_4$ or [^{35}S]-chlorosulfonic acid are most convenient.

1. With Adducts of Sulfur Trioxide

This is the first of the general methods and uses the sulfur trioxide adduct of a Lewis base as the sulfate donor. The most common of these adducts are pyridine-sulfur trioxide and *N*,*N*-dimethylaniline-sulfur trioxide, but those of triethylamine, dimethylformamide and dioxan, to name only a few, have been quite widely used. In general

Table 1
TYPES OF SULFATE ESTER AND RELATED COMPOUNDS FOUND IN BIOCHEMICAL SYSTEMS

Characteristic group	Type of compound	Naturally occurring examples	Examples formed in vivo from a xenobiotic
$\geq C.OSO_3^-$	Alkyl sulfates	Propan-2-yl sulfate	Dimetridazole → Hydroxydimetridazole sulfate
	Aryl sulfates[a]	Indoxyl sulfate	Salicylamide → Salicylamide sulfate
	Steroid sulfates		
	Phenols	Oestrone sulfate	Most steroids must be assumed to form sulfate esters in vivo.
	Primary alcohols	Cortisol 21-sulfate	
	Secondary alcohols	Cholesteryl sulfate	
	Carbohydrate sulfates[b]		
	Primary alcohols	Galactosamine 6-sulfate	No example known
	Secondary alcohols	Galactose 3-sulfate	
$>N.OSO_3^-$	Hydroxamic acid sulfates	Sinigrin[c]	*N*-Acetyl-2-aminofluorene → *N*-Acetyl-*N*-hydroxy-2-aminofluorene sulfate
$\geq C.SSO_3^-$	Thiosulfates	*S*-Sulfocysteine	No example known
$\geq C.NH.SO_3^-$	Sulfamates	2-Sulfoamino-2-deoxyglucose[d]	2-Naphthylamine → 2-Naphthyl sulfamate
$-O.PO(OH).OSO_3^-$	Sulfatophosphates	Adenosine 5′-sulfatophosphate	No example known

[a] Tyrosine *O*-sulfate occurs in some peptides such as fibrinopeptide B and caerulein.
[b] In animals, carbohydrate sulfates usually occur in more complex molecules such as glycosaminoglycans, sulfolipids, and glycosides (e.g., holuthurin A).
[c] Sinigrin and related mustard oil glycosides are of widespread occurrence in plants but no naturally occurring hydroxamic acid sulfate has yet been found in animals.
[d] In animals, occurs only in glycosaminoglycans.

terms, the reactivity of the adduct varies inversely with the strength of the base so that it is possible to moderate the fundamental reaction, that of the hydroxyl group with SO_3, to any desired extent by the choice of a suitable reagent, from the very reactive dimethylformamide-sulfur trioxide, through pyridine-sulfur trioxide, to the rather stable triethylamine-sulfur trioxide. However, as pointed out by Gilbert,[5] base strength is not the only factor which governs the reactivity of the adduct. Two variants of this general method are available, and both require strictly anhydrous conditions.

In the first, the adduct is formed by the reaction at 0°C, or less, of chlorosulfonic acid with the base in an inert solvent such as $CHCl_3$ or CS_2 after which the acceptor is added to the reaction mixture and the temperature allowed to rise. This method is simple, but the products may be contaminated with Cl^- which can be difficult to remove. Details can be found in the classical work of Burkhardt and Lapworth on the synthesis of aryl sulfates[6,7] and in more recent syntheses of the sulfates of hydroxypyridines.[8]

In the other general variant, the purified sulfur trioxide adduct is isolated and then used in the sulfation reaction. Obviously, in this case there is no possibility of contamination of the product with Cl^- and that with SO_4^{2-} is also generally less. The adducts are easily prepared[5] by the reaction of the base in an inert solvent, such as $CHCl_3$, with SO_3 or with chlorosulfonic acid; the latter method has the disadvantage that the hydrochloride of the base is produced in equivalent amounts, and although the major part of this can easily be removed, the traces which remain can sometimes cause

problems (see Section IIA6). Some sulfur trioxide adducts are commercially available. Examples of this type of sulfation are to be found in the synthesis of many types of steroid sulfate using triethylamine-sulfur trioxide,[9] and of carbohydrate sulfates[10] or the rather unstable sulfates of *N*-acetyl-*N*-hydroxyarylamines using pyridine-sulfur trioxide.[11,12]

2. With H_2SO_4 and a Carbodiimide

This is the second of the general methods, and although it has not been as widely used, it is potentially valuable. It uses H_2SO_4 as a sulfating agent in the presence of dicyclohexylcarbodiimide as a condensing agent. Synthesis of alkyl sulfates,[13] aryl sulfates,[13] carbohydrate sulfates,[14] steroid sulfates,[15] and oxime *O*-sulfates[13] have been described. More complex products such as pyrosulfate diesters are sometimes produced by this method.[13]

3. Miscellaneous Methods

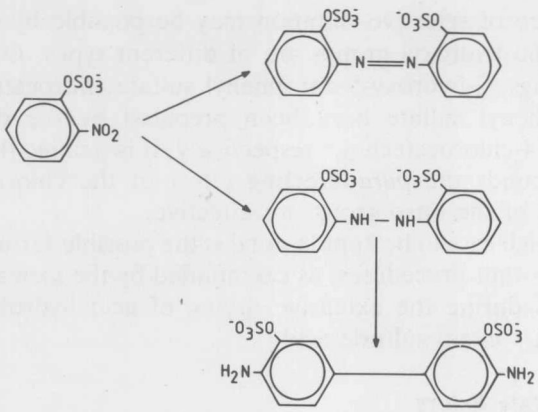
Many other methods of preparing sulfate esters are available. Direct sulfation with H_2SO_4 was classically used for the synthesis of simple alkyl sulfates,[1,2,16] but it is also useful for the preparation of the sulfate esters of amino compounds, for example, choline *O*-sulfate,[17] serine-*O*-sulfate,[18] tyrosine *O*-sulfate,[19] dopamine 3- and 4-*O*-sulfates,[20] and serotonin *O*-sulfate.[21] Chlorosulfonic acid has been used directly for the formation of hydroxylamine *O*-sulfate,[22] sulfamic acid for the synthesis of steroid sulfates[23] (see also Section IVD) and pyridinium sulfate plus acetic anhydride for the preparation of estrone sulfate.[24] The latter reaction has also been used in this laboratory for the preparation of a number of simple aryl sulfates. A sulfate group can also be transferred from a sulfate ester to an acceptor; steroid sulfates have been prepared by the oxidative transfer of the sulfate group from ascorbate 2-sulfate to the steroid,[25] while the Cu^{2+}-catalyzed transfer of sulfate from quinolinyl 8-sulfate has been used to prepare aryl sulfates and carbohydrate sulfates[26] (see also Section IIB2C).

4. Elb's Persulfate Oxidation

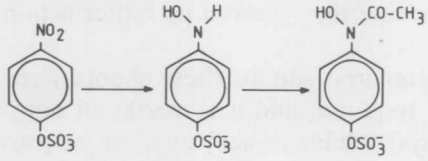
All of the above methods sulfate an existing hydroxyl group. In contrast, the Elb's persulfate oxidation introduces an $-O.SO_3^-$ group directly into an aromatic ring by treatment with peroxydisulfate under alkaline conditions. The oxidation of phenols has been most studied, and the reaction almost certainly involves an electrophilic attack of the phenoxide ion on the peroxide bond in the peroxydisulfate ion although earlier work suggested that the sulfate ion radical was the reactive species.[2] The method has been used for the synthesis of nitrocatechol[27] and nitroquinol[28] sulfates from 4- and 3-nitrophenol, respectively. Amines can also be oxidized, and methods have been described for the synthesis of several *o*-aminophenyl sulfates[29] as well as indoxyl sulfate.[30]

5. Indirect Methods

Some sulfate esters are best prepared by indirect methods, for example, the ketoxime *O*-sulfates by the reaction of hydroxylamine *O*-sulfate with the appropriate ketone.[31] Aminophenyl, aminocatechol, and aminoquinol sulfates are prepared by the reduction of the corresponding nitroaryl sulfates either by Fe^{2+} or by H_2/Pt.[7,32,33] More complex sulfate esters are obtained by reduction of nitroaryl sulfates in alkaline solution,[7] as is shown below for 2-nitrophenyl sulfate. This gives a mixture of azobenzene 2,2′-disulfate and hydrazobenzene 2,2′-disulfate, and the latter can be rearranged in acid to give 4,4′-diaminodiphenyl 3,3′-disulfate.



Reduction of nitroaryl sulfates with Zn gives a hydroxylamine, and this fact has recently been used[34] to prepare *N*-acetyl-*N*-hydroxy-4-aminophenyl sulfate by reduction of 4-nitrophenyl sulfate to the corresponding hydroxylamine which is then acetylated, as shown below.



6. Some Difficulties

The synthesis of sulfate esters is generally straightforward, and the products usually have the expected structures, but difficulties can arise when the parent alcohol is optically active because racemization may occur. Burwell[35,36] was the first to show that the extent of the racemization varied with the method of sulfation: sulfation of butan-2-ol with pyridine-sulfur trioxide, dioxan-sulfur trioxide, or sulfamic acid gave essentially complete retention of configuration, whereas sulfation with H_2SO_4 or chlorosulfonic acid gave extensive racemization, probably because of the existence of a complex equilibrium mixture containing butene. More recently, Matcham and Dogson[37] have confirmed these findings using a number of secondary alcohols, and they have further shown that the nature of the product obtained after sulfation with pyridine-sulfur trioxide depended upon how that reagent had been prepared. When prepared from pyridine and sulfur trioxide, the reagent gave complete retention of configuration, but when prepared from pyridine and chlorosulfonic acid,[3] racemization occurred. It was suggested that the difference in behavior was caused by the traces of pyridinium chloride which the latter reagent contained.[37] So far no similar phenomenon has been noted during the sulfation of steroids or carbohydrates. Matcham and Dodgson[37] have also shown that during the sulfation of scondary alcohols with H_2SO_4, migration of the hydroxyl group may occur, again probably because of the existence of an unsaturated intermediate.

The sulfation of polyhydroxy compounds can also pose problems which reach their peak in the synthesis of authentic monosaccharide sulfates. These can only be obtained [38] by the sulfation of suitably protected derivatives. Even with simple dihydric alcohols or phenols, difficulties can arise, and simply restricting the amount of sulfating agent to one molar proportion does not necessarily ensure the formation of a monosulfate

although some degree of selective sulfation may be possible by suitable choice of conditions[9,13] when the hydroxyl groups are of different types. On the other hand, despite these warnings, 2-hydroxy-5-nitrophenyl sulfate (nitrocatechol sulfate) and 4-chloro-2-hydroxyphenyl sulfate have been prepared by the direct sulfation of 4-nitrocatechol[39] and 4-chlorocatechol,[40] respectively. It is striking that in the synthesis of these two compounds the *para*-directing effect of the chlorine atom and the *meta*-directing effect of the nitro group are effective.

Finally, a point which should be kept in mind is the possible fortuitous formation of sulfate esters during other procedures, as exemplified by the formation of serine and threonine *O*-sulfates during the extensive drying of acid hydrolystates of histone sulfates[41] which must contain sulfuric acid.

B. Properties of Sulfate Esters

Sulfate esters are normally obtained as salts, internal salts in suitable cases.[8,26] The corresponding free acids, the X hydrogen sulfates, are generally unstable and cannot be isolated although alkyl hydrogen sulfates, including at least some carbohydrate sulfates, can be obtained in solution, most simply by passage of a solution of the salt through the H^+ form of a strong cation exchange resin. The free acids are strong acids, but their strengths have not been determined, although it was long ago suggested[42] that they were stronger than H_2SO_4 because they showed no buffer action in 0.25 *M* HCl, whereas K_2SO_4 did.

The most commonly prepared salts are those of potassium, but those of other metals are easily obtainable if required, and it is worthy of note that the barium salts are generally (but not always) soluble in acid solution, so providing a useful distinction between $R.OSO_3^-$ and SO_4^{2-}. Again in general, the alkali metal salts of sulfate esters are soluble only in polar solvents—water, lower alcohols, dimethylformamide, etc. A useful solvent is butan-l-ol because it can be used to extract many sulfate esters, especially aryl sulfates and steroid sulfates, from aqueous solution.

The salts of organic bases, unlike the alkali metal salts, frequently have sharp melting points and some are soluble in nonpolar solvents. The methylene blue salts of many sulfate esters are soluble in $CHCl_3$ and this is the basis of a quantitative method for their determination[3] and for their detection on paper[43] and thin-layer[44] chromatograms (see also Section VI). The pyridinium salts of several steroid sulfates are also soluble in $CHCl_3$.[45] Other commonly used bases are 4-toluidine,[46] the aminoacridines,[40] and, especially for carbohydrate sulfates, brucine.[38] This solubility in nonpolar solvents of the salts of sulfate esters with organic bases should be kept in mind when isolating sulfate esters from natural sources because ion pairs, soluble in say $CHCl_3$, may be formed with naturally occurring cations. For example, dehydroepiandrosterone sulfate, and so presumably other steroid sulfates, form complexes with cationic detergents and with phosphatides which are soluble in $CHCl_3$; obviously this phenomenon could, if unsuspected, cause a loss of sulfate ester.[47] It is of interest that the converse situation may also be important because there are indications of an interaction in vivo between the sulfolipid, cerebroside sulfate (containing galactose 3-sulfate residues) and biogenic amines.[48]

Because sulfate esters are generally prepared as the barium, potassium, or sodium salts, there is almost no information on any influence the cation might have on the behavior of the ester. This influence may not be negligible, as was shown by the early studies of Sobel et al. They showed[49] that while the calcium and ferrous salts of cholesteryl sulfate required heating at 80 to 110°C to form dicholesteryl ether, the aluminium salt slowly underwent the same reaction at room temperature.

There has been no tabulation of the properties of sulfate esters in general but a useful summary of the physical characteristics of steroid sulfates is available.[50]

1. Spectral Properties

The spectral properties of sulfate esters mainly reflect the structure of the organic part of the molecule. The visible and ultraviolet spectra usually show little of interest although they can be valuable for the identification and determination of specific esters. Almost the only generalization which can be made is that aryl sulfates show a decreased λ_{max} and a slightly decreased ϵ_{max} when compared with the parent phenol.[8,51] These differences are particularly obvious in alkaline solution, and the striking difference between, say, the yellow 4-nitrophenolate ion and the colorless 4-nitrophenyl sulfate has many analytical applications.

Measurements of specific rotation have been useful in characterizing carbohydrate sulfates[38] and secondary alkyl sulfates.[52] With the latter there are surprisingly large solvent effects: changing the solvent from water to 80% ethanol changed the sign of the rotation, a phenomenon which, until recognized, caused some confusion.

Infrared spectra can be informative because of the absorptions due to the sulfate group itself, principally those assigned to S–O bond stretching in the 1210- to 1250-cm^{-1} region and to C–O–S vibrations in the 800- to 850-cm^{-1} region. Both alkyl[53] and aryl[8,54] sulfates have been examined, and a considerable amount of interest has been shown in the carbohydrate sulfates because it was at one time considered[55] that the position of the absorption in the 1240-cm^{-1} region could be used to differentiate between the sulfate esters of primary, secondary axial, and secondary equatorial hydroxyl groups. This is not the case, and it is now obvious that any aglycone or substituent on the sugar may profoundly influence the position of the C–O–S absorption.[56]

Nuclear magnetic resonance spectra are again of interest primarily with regard to the structure of the organic part of the molecule, and particularly with the localization of the sulfate group in polyhydroxy compounds. The sulfate esters of polyhydroxy phenols[54] and of glycosides[56] have been examined by PMR, but much more useful information is available from ^{13}C–NMR spectra. In polyhydroxy phenols,[54] sulfation causes a downfield shift, with respect to tetramethylsilane, of 2.8 to 5.8 ppm in the signal from the carbon atom carrying the sulfate group and larger upfield shifts of 5.4 to 7.8 ppm in the signals from the carbon atoms *ortho* and *para* to this. In monosaccharide sulfates,[56-59] sulfation is accompanied by a marked downfield shift of between 6.2 and 9.5 ppm in the signal from the carbon atom carrying the sulfate group compared with up-field shifts of 1.1 to 2.5 ppm in the signals from the adjacent carbon atoms. Some data are given in Table 2, which also shows that it is possible to distinguish between the α and β anomers of the carbohydrate sulfates. It seems safe to say that in the future, ^{13}C–NMR will be the method of choice for the characterization of the sulfate esters of polyhydroxy compounds.

Mass spectrometry has been little used in studies of sulfate esters because of the involatility of the usual salts, but reports have appeared of the application of field desorption mass spectrometry to the alkali metal salts of some alkyl and steroid sulfates[37,60] and of electron impact mass spectrometry of aryl sulfates after their conversion to the corresponding alkylaryl diesters.[61] Usov et al[62] have used mass spectrometry to locate the substituent in a series of amidosulfates of D-galactose, and this suggests that a similar technique might be a useful adjunct to the ^{13}C–NMR methods described above for the characterization of monosaccharide sulfates. Some further information is to be found in Section IIB4.

2. Hydrolysis

Sulfate esters are hydrolyzed in acid, neutral, and alkaline solutions, but the rates of