Collisionally-induced dissociation mass spectra of organic sulfate anions

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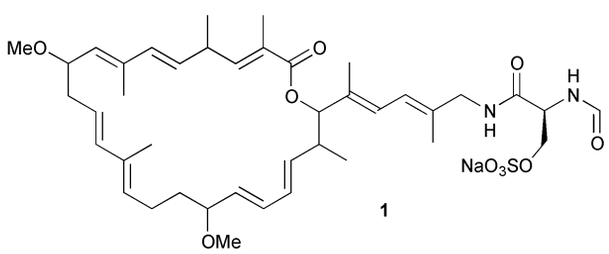
The collisionally-induced dissociation mass spectra of a variety of organic sulfate ester anions are described and mechanistically rationalized. A cyclic *syn*-elimination pathway, analogous to that of the Cope elimination, is postulated for the commonly observed formation of bisulfate anion (HSO_4^-, *m*/*z* 97). Deuterium labeling experiments confirm that the proton transferred to oxygen during bisulfate elimination normally originates from the C-2 position, although examination of the spectra of polyfunctional steroids reveals that the proton abstracted may originate from more distant sites as well. Adamantyl, phenyl, and vinyl sulfate anions, which do not readily lend themselves to a cyclic *syn*-elimination, do not give rise to an *m*/*z* 97 ion. Instead, these sulfates undergo both heterolytic and homolytic S–O bond cleavages to yield an *m*/*z* M − 80 anion, resulting from loss of neutral SO_3, as well as an ion at *m*/*z* 80, corresponding to $SO_3^{-\bullet}$, respectively. Sulfates that can give rise to a resonance stabilized radical by homolytic C–O bond fission, as exemplified by benzyl and linalyl sulfates, can be recognized by the formation of an *m*/*z* 96 ($SO_4^{-\bullet}$) ion.

Introduction

Sulfate esters constitute a widespread and highly diverse group of natural and non-natural compounds. The resonance-stabilized nature of sulfate mono-ester anions underlies their unique chemistry, providing an electrostatic component to specific interactions without giving rise to significantly basic or nucleophilic behavior. The industrial importance of anionic surfactants such as sodium dodecyl sulfate (SDS) has tended to overshadow the significance of the increasing recognized number of biologically active natural products that contain a sulfate moiety. While once believed to occur exclusively as metabolites of marine organisms[1] (*i.e.* iejimalide D, **1**, from the tunicate *Eudistoma cf. rigida*,[2] and squalamine, **2**, from the dogfish shark[3]), sulfate esters are now known from many other sources (*e.g.* uzarigenin 3-sulfate, **3**, from the Ranunculaceae plant *Adonis aleppica*[4]).[5] In addition, knowledge of the enzymes that catalyze sulfate ester synthesis has grown exponentially during the last decade.[6] The formation of sulfate esters[7] is an important step in the elimination[8] or bioactivation[9] of xenobiotics and drugs, and in the biotransformations of many hormones and neurotransmitters.[6a,b,c,e] Recent studies of sulfated carbohydrates have revealed that many of these compounds populate extracellular spaces, and mediate a diverse range of events that contribute to intercellular recognition in both normal and pathological processes.[10]

Since encountering the first glycosylated nucleoside sulfate ester HF-7 (**4**) in the venom of a funnel-web spider,[11] we became interested in the possibility that similar anionic sulfate esters might have gone undetected in neurotoxic venoms. The ideal technique for searching for such compounds is clearly mass spectrometry. The facile ionization of sulfates obviously renders them amenable to negative-ion mass spectrometry. Particularly in conjunction with electrospray ionization (ESI) and liquid chromatography (LC), mass spectrometry (MS) has already provided a powerful approach to the characterization of sulfated compounds in biological fluids.[12–16] Nevertheless, although several interesting features have been noted in the mass spectra of these sulfate esters,[12,14,15,17] little attention has been paid to the details of their fragmentation mechanisms. A prominent product ion peak clearly attributable to HSO_4^- has been observed at *m*/*z* 97 in most spectra of organic sulfates.[17] The formation of this anion requires the transfer of a proton to oxygen, in addition to the cleavage of a C–O bond. The origin of this transferred proton, however, has not been established,



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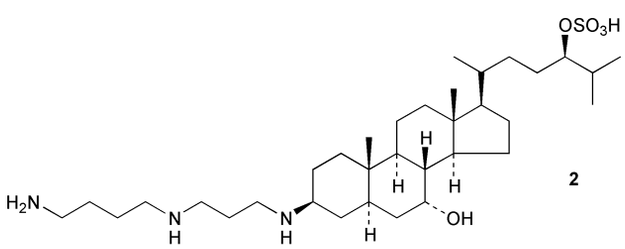

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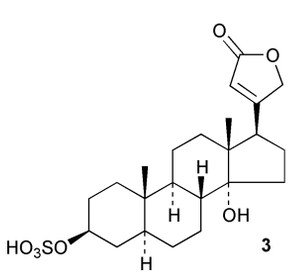

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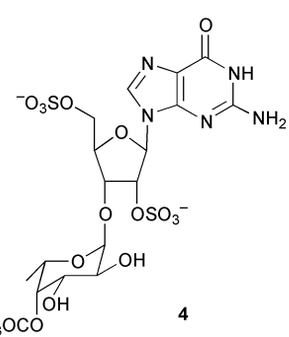

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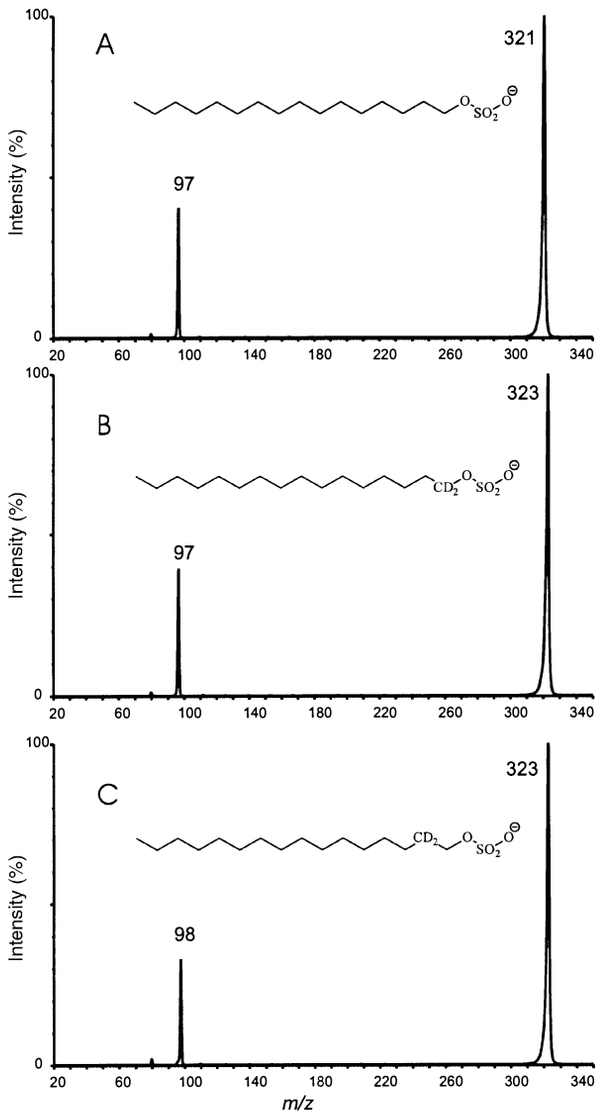


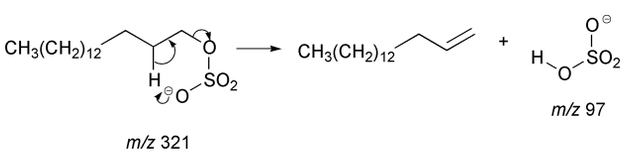
Fig. 1 Product ion spectra of molecular anions of hexadecyl sulfate (A), [1,1-$^2H_2$]hexadecyl sulfate (B), and [2,2-$^2H_2$]hexadecyl sulfate (C) (collision energy 45 eV; cone voltage 56 V).

although the hydrogen atom geminal to the sulfate group has been suggested as its source.[12] We here report results obtained with a number of natural and synthetic sulfates subjected to electrospray ionization and MS-MS experiments under collisionally-induced dissociation (CID) conditions. These results clarify the mechanism of bisulfate† elimination as well as the other fragmentation patterns characteristic of this important group of compounds.

Results and discussion

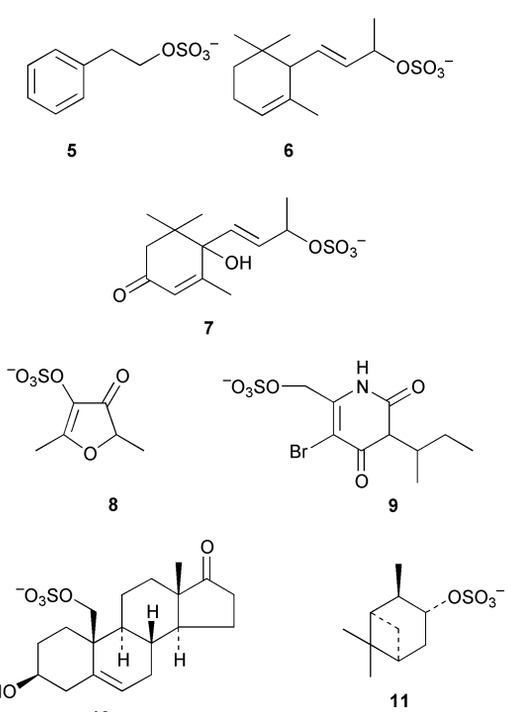
Compared to high-energy negative-ion CID mass spectra of organic sulfate esters,[18] those spectra generated by low-energy collisions are relatively simple.[19] For example, the negative-ion CID spectrum of dodecyl sulfate anion shows only the parent ion at *m/z* 265 (100%), a prominent product ion at *m/z* 97 (17%), and a very weak signal at *m/z* 80 ($SO_3^{-\bullet}$, <1%). The *m/z* 97 peak is generally understood to represent the HSO_4^- anion. In accord with this assignment, we have observed the corresponding formation of an *m/z* 99 ($H^{34}SO_4^-$) ion from the ^{34}S-isotopomer of dodecyl sulfate.

To determine whether a geminal C-1 proton is utilized in the formation of bisulfate anion, we synthesized and recorded the spectrum of [1,1-$^2H_2$]hexadecyl sulfate. A comparison of the CID product-ion spectra derived from *m/z* 323 and 321, from the quasi-molecular ions of [1,1-$^2H_2$]hexadecyl sulfate and hexadecyl sulfate, respectively, showed the formation of an *m/z* 97 ion in both cases (Fig. 1A and 1B). Since an *m/z* 98 ion was not observed for [1,1-$^2H_2$]hexadecyl sulfate, it is evident that the C-1 deuterium atoms were not abstracted. On the other hand, the product ion spectrum of *m/z* 323 from [2,2-$^2H_2$]hexadecyl sulfate does show a peak at *m/z* 98 (Fig. 1C), indicating that a deuteron from the C-2 position is transferred specifically. Consequently, the fragmentation process is best represented as a concerted *syn*-elimination (Scheme 1) similar to the mechanism for the Cope amine *N*-oxide elimination and other pyrolytic eliminations that occur in the gas phase.[20]



Scheme 1

A literature survey of sulfate ester mass spectra supports this conclusion. The negative-ion ESI mass spectra of sulfates of 2-phenylethanol (**5**), α-ionol (**6**), and vomifoliol (**7**), all of which



possess protons in the C-2 position, show a peak for the bisulfate anion, whereas those of benzyl sulfate, 2,5-dimethyl-3-oxo-2,3-dihydrofuran-4-yl sulfate (**8**),[15] the radio-labeled bromasil metabolite **9**,[16] and 3β-hydroxy-17-oxoandrost-5-en-19-yl sulfate (**10**),[14] lacking C-2 protons, do not. Our experimental results with 2-methyl-2-pentyl- and 3-methyl-3-pentyl sulfates showed the formation of the *m/z* 97 peak as the major fragment, confirming that the protons in the position C-1 are not involved in the formation of HSO_4^-.

An important requirement for facile HSO_4^- elimination is the ability of the resulting neutral product to accommodate a double bond between C-1 and C-2. Thus, the spectrum of isopinocamphyl sulfate (**11**) shows the anticipated *m/z* 97 peak, whereas that of adamantyl sulfate (**12**) does not, since the adamantyl ring system cannot accommodate a double bond in its rigid skeleton[21] (Fig. 2). Instead, an ion at *m/z* 151 is formed

† The IUPAC name for bisulfate is hydrogen sulfate.

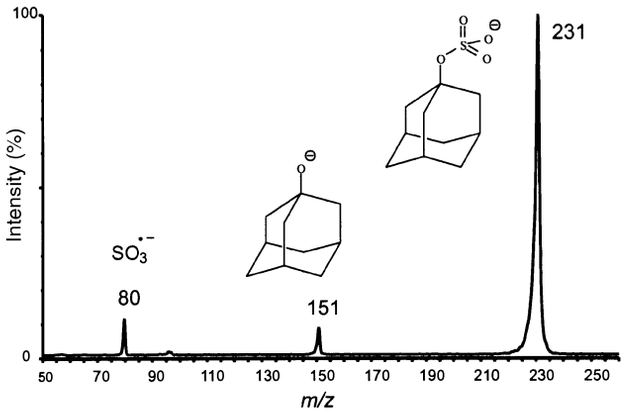
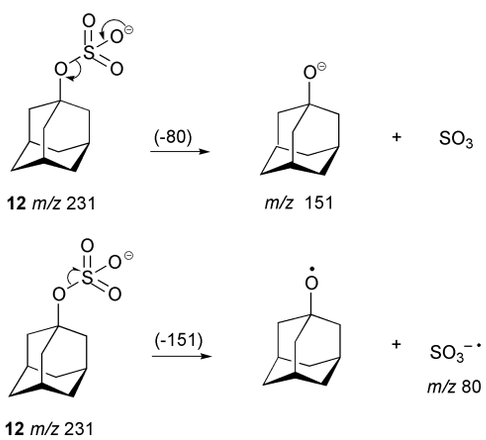


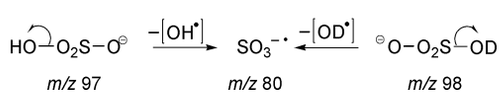
Fig. 2 Product ion spectrum of quasi-molecular anion of adamantyl sulfate **12** (collision energy 66 eV; cone voltage 60 V).

by heterolytic cleavage of an S–O bond resulting in loss of SO_3 from the *m/z* 231 ion, while an *m/z* 80 ion ($SO_3^{-\bullet}$) originates from a homolytic cleavage of the same bond (Scheme 2).



Scheme 2

This homolytic process is not unique to **12**. The low intensity peak observed at *m/z* 80 in many sulfate ester spectra (Fig. 1) originates from homolytic fission of an S–O bond, either directly from the quasi-molecular anion, or indirectly from the bisulfate anion itself as depicted in Scheme 3. Evidence for this fragmentation mechanism was obtained by recording product ion spectra of bisulfate anions originating from hexadecyl sulfate, [1,1-$^2H_2$]hexadecyl sulfate, and [2,2-$^2H_2$]hexadecyl sulfate (Fig. 3). The formation of an *m/z* 80 ion from both HSO_4^- and DSO_4^- by loss of a hydroxyl or deuteroxyl radical, respectively, confirms this proposal (Scheme 3).



Scheme 3

Steroidal sulfates, which are not able to activate the appropriate steroid receptors, are of particular interest since they represent an inactive form of transport from which the hormone can be regenerated in the target tissue.[6c] We have therefore paid particular attention to the CID spectra of this group of biologically important sulfates. The formation of HSO_4^- has been observed in the spectra of many steroidal sulfates.[12,19,22,23] For example, the negative ion ESI spectrum obtained from dehydroepiandrosterone sulfate (**13**) shows two major peaks: the most abundant peak at *m/z* 97, resulting from the loss of a bisulfate anion from ring A, and the molecular ion at *m/z* 367 (Scheme 4).

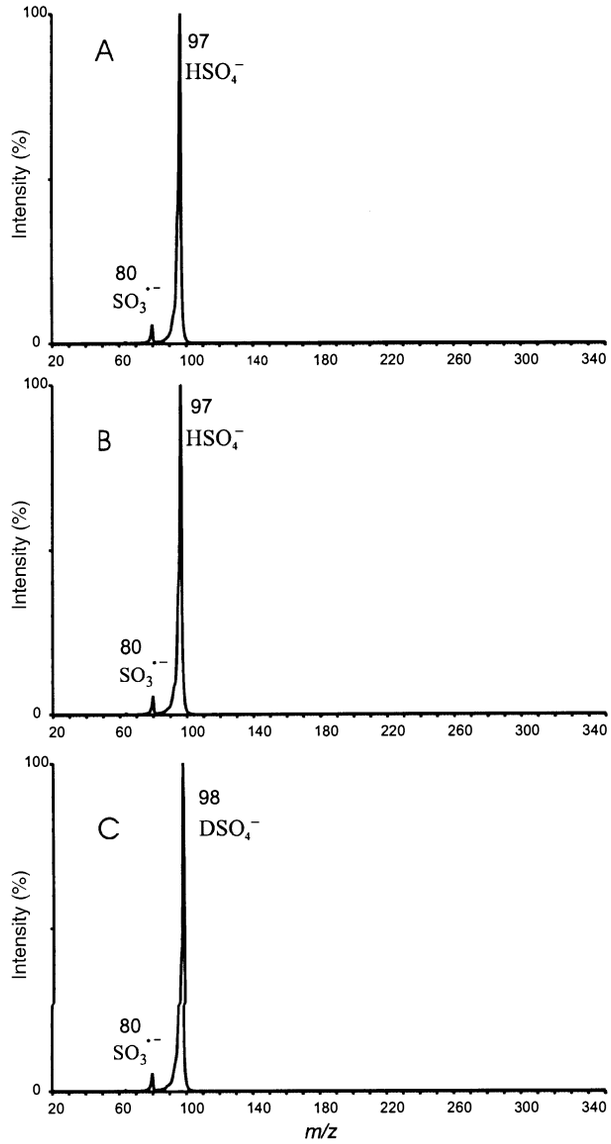
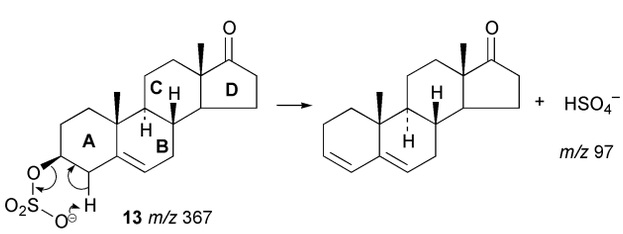


Fig. 3 Product ion spectra of bisulfate anions derived from hexadecyl sulfate (*m/z* 97) (A), [1,1-$^2H_2$]hexadecyl sulfate (*m/z* 97) (B), and [2,2-$^2H_2$]hexadecyl sulfate (*m/z* 98) (C) (collision energy 45 eV; cone voltage 56 V).



Scheme 4

An interesting conformational effect is revealed in the CID spectra derived from the *m/z* 467 ions of the epimeric 5α-cholestan-3β-yl sulfate (**14a**) and 5α-cholestan-3α-yl sulfate (**15a**). Both **14a** and **15a** show the formation of the bisulfate anion; however, the intensity of the *m/z* 97 peak from the 3α-sulfate is considerably greater than that from the 3β-epimer (Fig. 4A). The observed differences appear to reflect the greater relief of conformational strain upon the elimination of the axial 3α-substituent. Similar intensity differences have been observed in the fast atom bombardment ionization spectra of **14a** and **15a**.[18] Based on these observations, it is likely that the stereochemistry of an unknown axial or equatorial steroidal sulfate can be determined if both epimers are available.

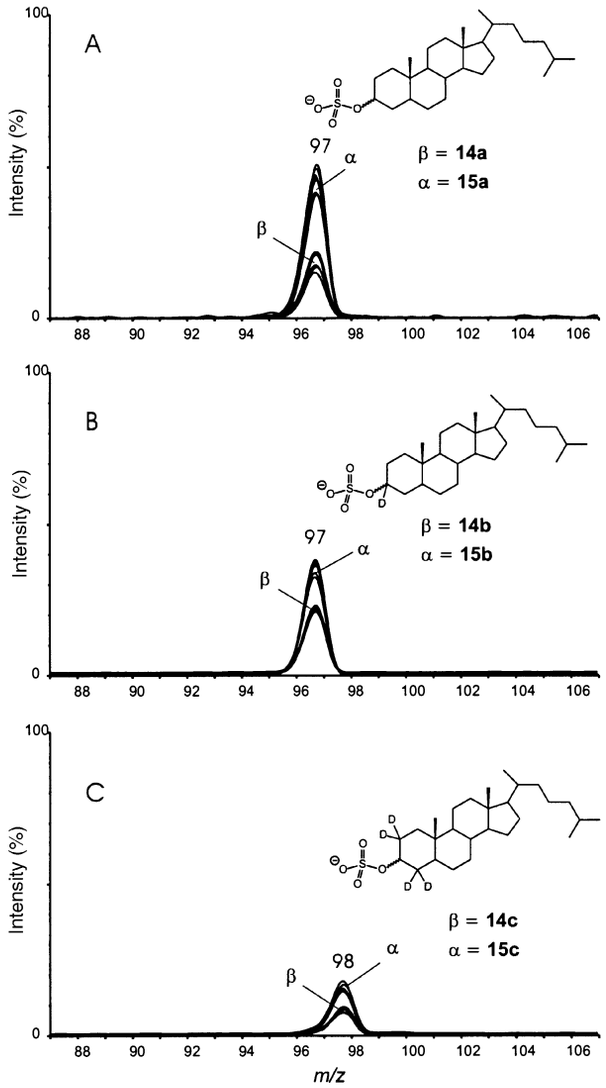
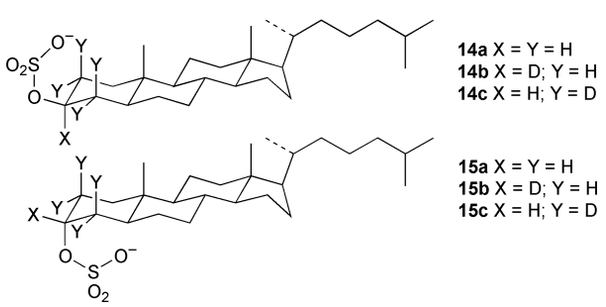
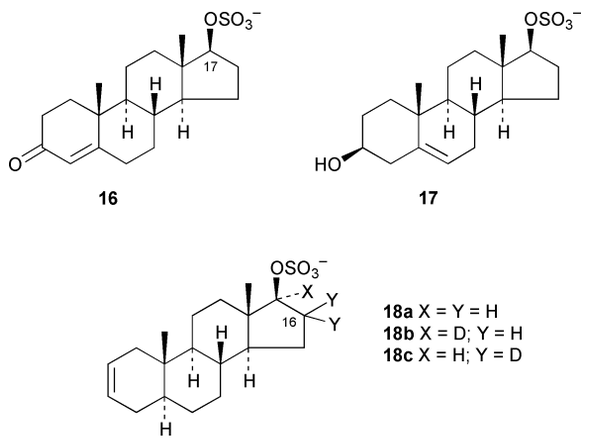


Fig. 4 Product ion spectra recorded by repeated experiments under identical conditions of *m/z* 467 of 5α-cholestan-3β-yl sulfate (**14a**, lower six traces) and 5α-cholestan-3α-yl sulfate (**15a**, upper six traces) (A), *m/z* 468 of 5α-[3α-$^2H_1$]cholestan-3β-yl sulfate (**14b**, lower six traces) and 5α-[3β-$^2H_1$]cholestan-3α-yl sulfate (**15b**, upper six traces) (B), and *m/z* 471 of 5α-[2,2,4,4-$^2H_4$]cholestan-3β-yl sulfate (**14c**, lower six traces), and 5α-[2,2,4,4-$^2H_4$]cholestan-3α-yl sulfate (**15c**, upper six traces) (C). See text for experimental details (collision energy 40 eV). All spectra were normalized to the intensity of the parent ion (not shown).

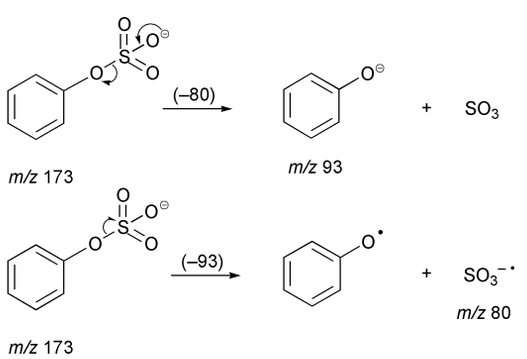


The product ion spectra derived from *m/z* 468 of 5α-[3β-$^2H_1$]cholestan-3β-yl sulfate (**14b**) and 5α-[3α-$^2H_1$]cholestan-3α-yl sulfate (**15b**) show similar intensity differences for the *m/z* 97 peak (Fig. 4B); moreover, a secondary kinetic isotope effect is observed, since the relative intensities of the *m/z* 97 ion obtained from this experiment are lower than those recorded from their non-deuteriated analogs. In accordance with expectation, the product ion spectra of *m/z* 471 for both 5α-[2,2,4,4-$^2H_4$]cholestan-3β-yl sulfate (**14c**) and 5α-[2,2,4,4-$^2H_4$]-cholestan-3α-ol sulfate (**15c**) give rise to DSO_4^- (*m/z* 98) (Fig. 4C). In this case, the relative intensities of the *m/z* 98 ion are much lower than those recorded for the *m/z* 97 ion from **14a**, **15a**, **14b** and **15b** due to the anticipated primary kinetic isotope effect.

Not surprisingly, the formation of the bisulfate anion is observed also in the spectra of compounds with a sulfate group attached to a five-membered ring. Reported spectra for the sulfate of testosterone (**16**),[22] and 3β-hydroxy-5α-androst-5-en-17β-yl sulfate[14] (**17**) show the expected *m/z* 97 peak. Mass spectra of the sulfates of 5α-androst-2-en-17β-ol (**18a**), 5α-[17α-$^2H_1$]-androst-2-en-17β-ol (**18b**), and 5α-[16,16-$^2H_2$]androst-2-en-17β-ol (**18c**) (Fig. 5) confirm that the proton transfer takes place from C-16.

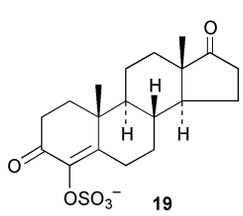


Neither aryl nor vinyl sulfate esters, in which a C-2 proton is attached to an sp^2 carbon, undergo bisulfate elimination.[12,17,24] Thus, the spectrum of phenyl sulfate fails to exhibit a signal at *m/z* 97 (Fig. 6A). Instead, a peak at *m/z* 93 for a loss of SO_3, and at *m/z* 80 for $SO_3^{-\bullet}$ are both observed (Scheme 5).



Scheme 5

Spectra published for 2,5-dimethyl-3-oxo-2,3-dihydrofuran-4-yl sulfate (**8**),[15] 3,7-dioxoandrost-4-en-4-yl sulfate (**19**),[25] and sulfated tyrosine derivatives of peptides[26] suggest that the loss of SO_3 from the quasi-molecular anion is a common feature observed in the spectra of vinyl and aryl sulfates.



Estron-3-yl 3-sulfate (**20**) behaves analogously, losing SO_3 to produce a phenoxyl anion at *m/z* 269 (**21**). While the observation that spectra of aryl sulfates fail to show a *m/z* 97 peak

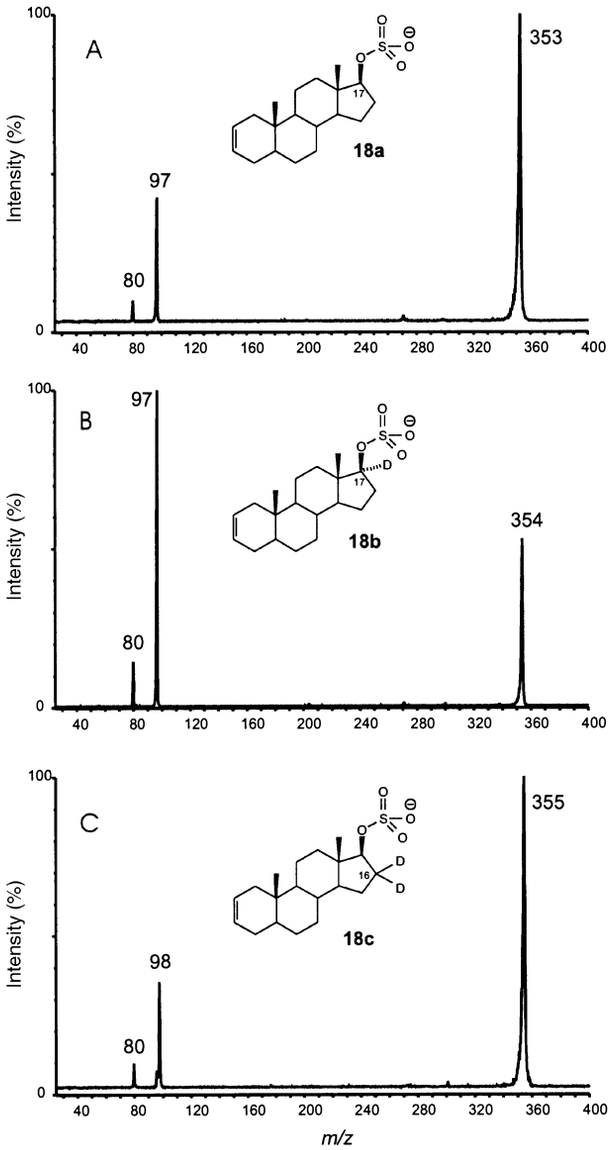


Fig. 5 Product ion spectra of quasi-molecular ions of sulfates of 5α-androst-2-en-17β-ol, **18a** (A) (collision energy 56 eV; cone voltage 39 V), 5α-[17α-$^2H_1$]androst-2-en-17β-ol, **18b** (B) (collision energy 52 eV; cone voltage 56 V) (B), and 5α-[16,16-$^2H_2$]androst-2-en-17β-ol, **18c** (C) (collision energy 45 eV; cone voltage 56 V).

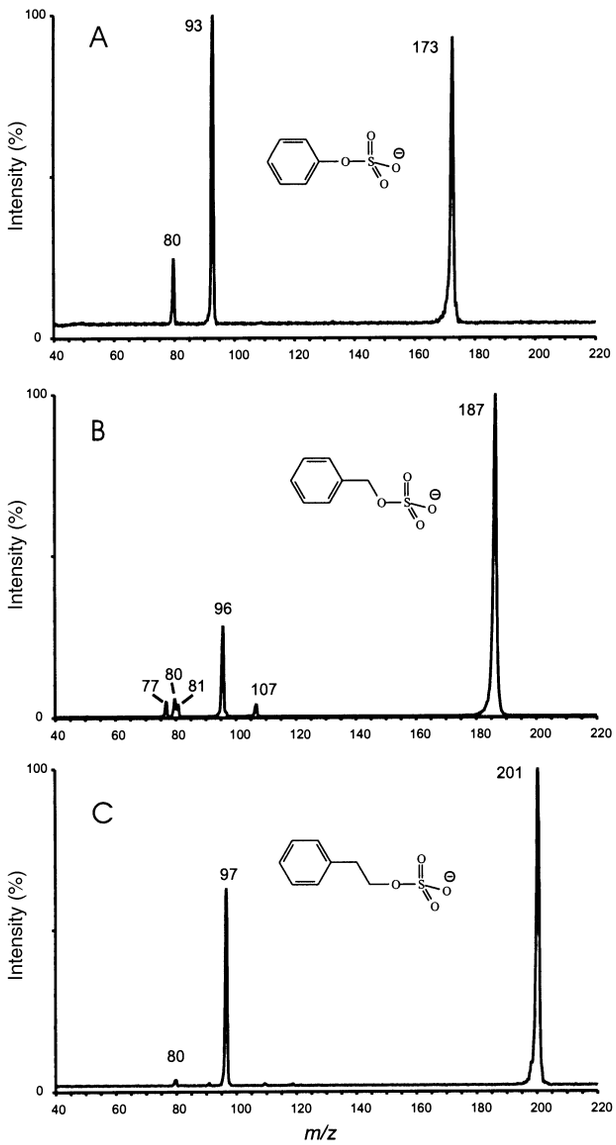
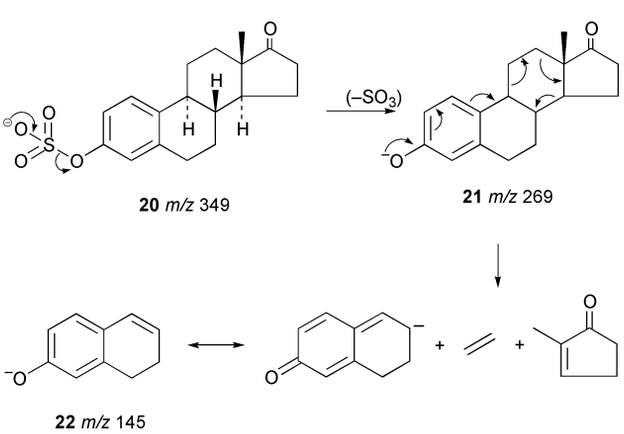


Fig. 6 Product ion spectra of quasi-molecular anions of phenyl sulfate (A) (collision energy 26 eV; cone voltage 42 V), benzyl sulfate (B) (collision energy 23 eV; cone voltage 24 V), and 2-phenylethyl sulfate (C) (collision energy 26 eV; cone voltage 25 V) anions.

has been attributed to the lack of a geminal proton,[12,14] this behavior appears rather to reflect the difficulty in forming a benzyne. Interestingly, at higher collision energies, the *m/z* 269 (**21**) ion derived from **20** undergoes a cycloreversion of the C ring to yield a bicyclic ion at *m/z* 145 (**22**), which is diagnostic for a sulfate group attached to a steroidal aromatic A ring (Scheme 6) as noted previously for 17β-estradiol 3-sulfate,[12] and estriol 3-sulfate.[12b]

Similarly, a sulfate group attached to the D ring (Scheme 7) can be recognized by the characteristic ion *m/z* 177 (**24**), which is observed in the spectra of the sulfate derivative of boldenone (17β-hydroxyandrosta-1,4-dien-3-one)[12a] (**23**) and the sulfate of testosterone[22] (**16**). While the formation of **24** requires cleavage of ring C, we do not have sufficient data to justify a charge-remote or a charge-mediated mechanism analogous to that proposed in Scheme 6.

These generalizations can now be applied to interpret the spectrum of 17β-estradiol 3,17-disulfate,[12] which shows signals at *m/z* 215 for the doubly-charged anion (**25a**), and *m/z* 431 for the singly-charged anion (**25b**) (Fig. 7A). The product ion spectrum of **25a** shows a loss of SO_3 to produce the doubly-charged ion **26** at *m/z* 175 (Fig. 7C) (rather than the previously



Scheme 6

proposed $C_6H_7O_4S$)[12b] whose nature is confirmed by the observation that its isotopic peaks are separated by 0.5 mass units (Fig. 7C inset). A precursor ion experiment confirmed that **26** originated from the *m/z* 215 ion (**25a**). On the other hand, the S–O bond can also be cleaved homolytically, to form two

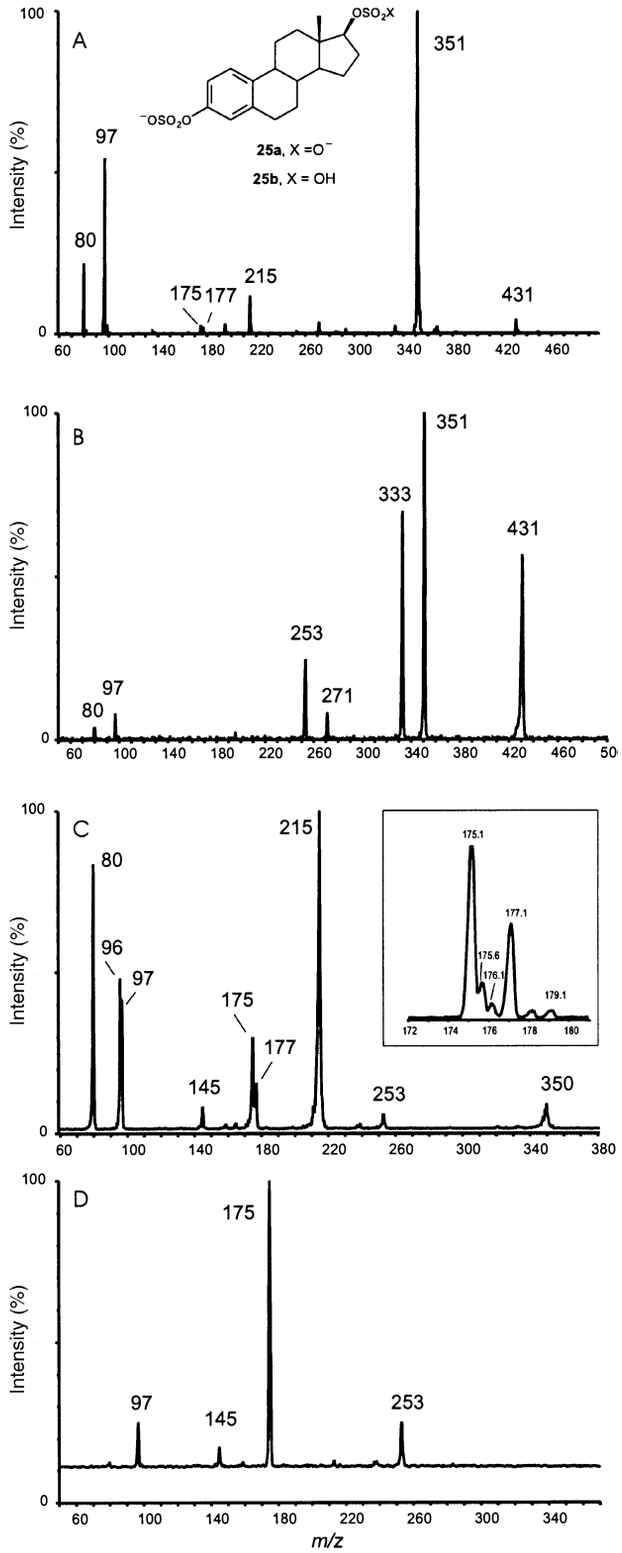
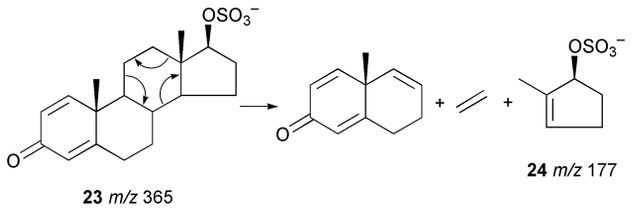


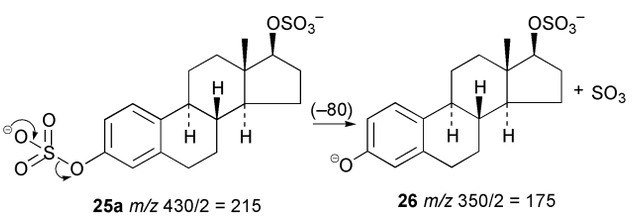
Fig. 7 Negative-ion ESI spectrum of 17β-estradiol 3,17-disulfate (cone voltage 40 V) (A). Product ion spectrum of **25b**, *m/z* 431 (collision energy 44 eV; cone voltage 17 V) (B). Product ion spectra of doubly-charged ions **25a**, *m/z* 215 (collision energy 44 eV; cone voltage 29 V) (C) and **26**, *m/z* 175 (collision energy 10 eV; cone voltage 46 V) (D). The inset in C shows an expansion of the *m/z* 175 region, recorded under source CID conditions, depicting the resolution of the isotopic peaks. (Note: the sample used was prepared from estradiol and may contain monosulfates as impurities.)

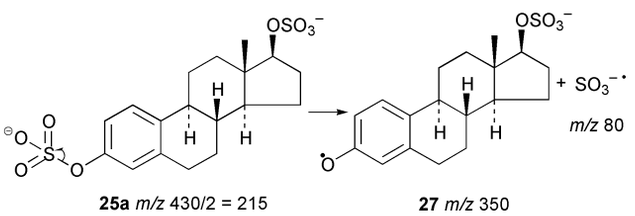
separate anion radicals at *m/z* 350 (**27**) and *m/z* 80, respectively (Scheme 8).

At higher collision energies, the doubly-charged fragment ion of *m/z* 175 (**26**) undergoes an HSO_4^- elimination, producing

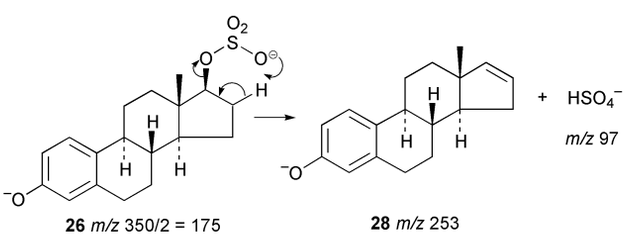


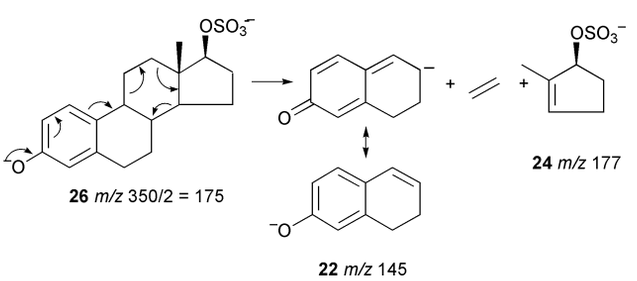
Scheme 7





Scheme 8





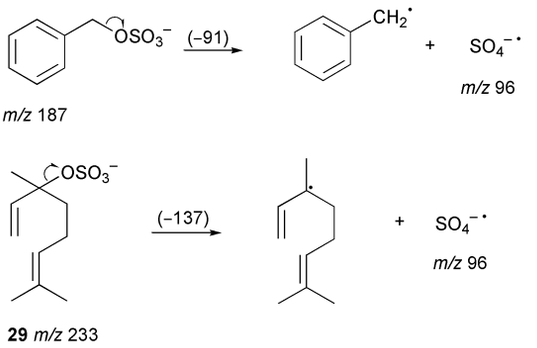
Scheme 9

an *m/z* 253 ion (**28**), as well as a charge-directed cycloreversion of the C ring, yielding *m/z* 145 (**22**) and *m/z* 177 (**24**) ions (Scheme 9, Fig. 7D). A product ion scan of **24** showed that it fragments further to form the *m/z* 97 anion, whereas a precursor ion scan confirmed that it can originate from both the *m/z* 215 (**25a**) and *m/z* 350 (**27**) ions.

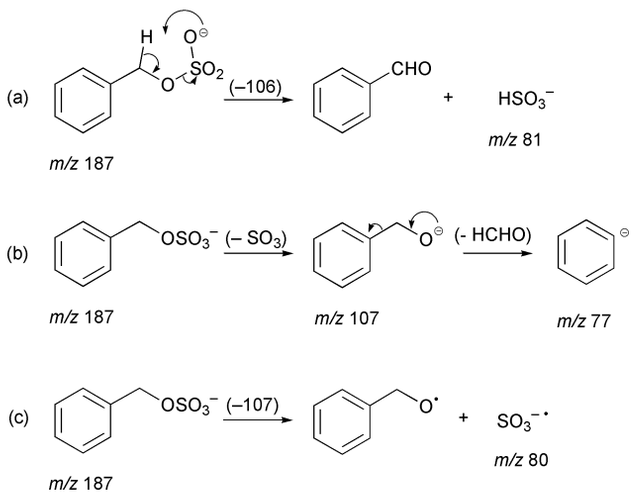
The CID spectrum of the singly-charged anion of 17β-estradiol 3,17-disulfate (**25a**, *m/z* 431), showed a loss of SO_3 and the elements of H_2SO_4, suggesting that a non-ionized monosulfate ester can also undergo a cyclic *syn*-elimination (Fig. 7B).

The fragmentation of benzyl sulfate is particularly interesting (Fig. 6B). The main fragment ion is observed at *m/z* 96, corresponding to the loss of a resonance-stabilized benzyl radical to give $SO_4^{-\bullet}$. In fact, the formation of $SO_4^{-\bullet}$ also appears to be characteristic for allylic sulfates that can yield stabilized radicals as products, since the major peak in the product ion spectrum of linalyl sulfate (**29**, Scheme 10) is also at *m/z* 96.

Pathways for the formation of minor ions from benzyl sulfate are rationalized in Scheme 11. For example, the elimination of a



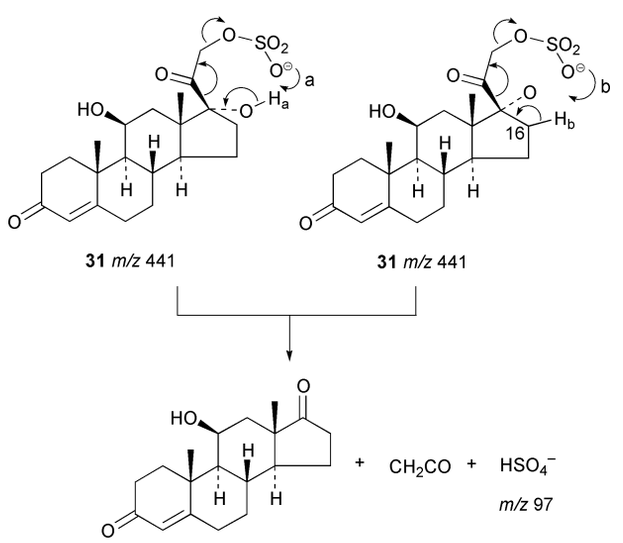
Scheme 10



Scheme 11

C-1 benzylic proton gives rise to HSO_3^- (*m/z* 81) by loss of a neutral molecule of benzaldehyde.

Recording mass spectra of sulfate esters with hydroxy groups proximal to the sulfate moiety revealed that alternative mechanisms for transfer of a proton to produce HSO_4^- are possible. The CID spectra of the *m/z* 439 ion of cortison-21-yl sulfate (**30**) and the *m*/z 441 ion of hydrocortison-21-yl sulfate (**31**) showed the *m/z* 97 product ion in spite of the absence of a C-2 proton (Fig. 8A and 8C). The proton transfer mechanism depicted in pathway a (Scheme 12) with the elimination of two



Scheme 12

neutral molecules, a ketene and a ketone, entails properties of heterolytic fragmentations described by Grob and Schiess.[27] The mechanism we propose is supported by the observation

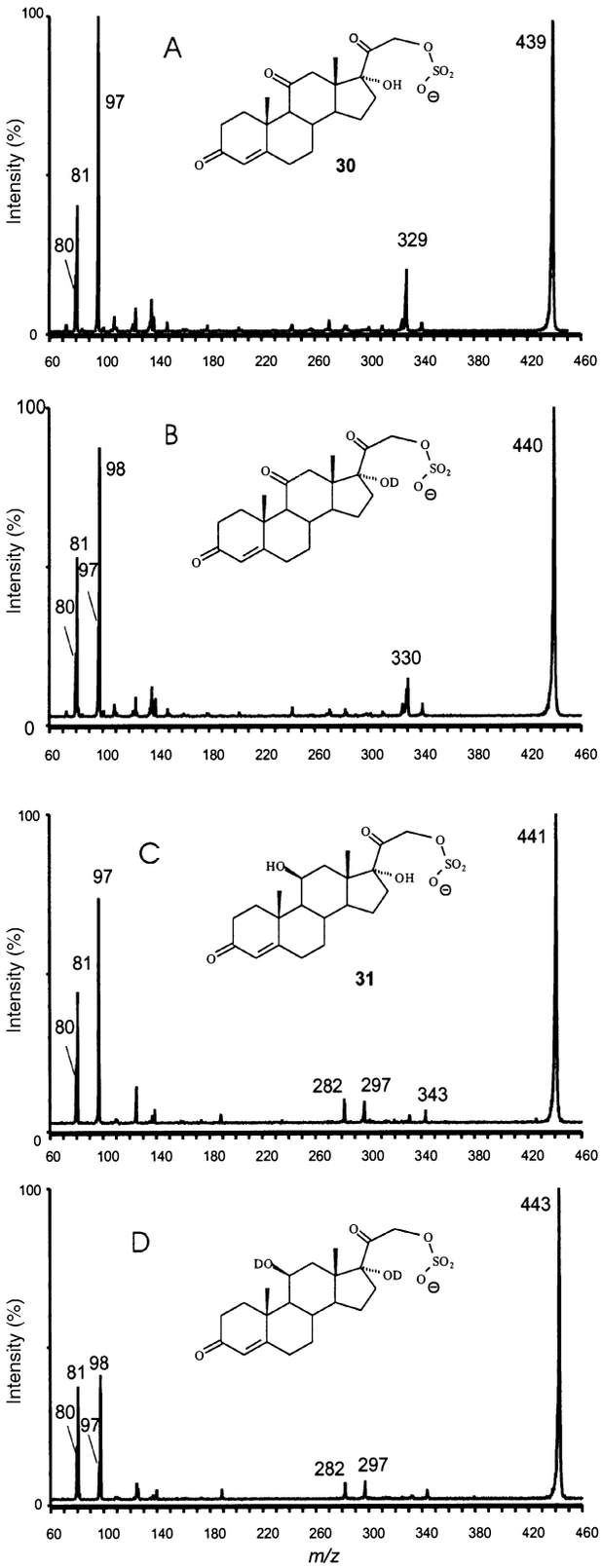


Fig. 8 Negative-ion CID spectra of cortison-21-yl sulfate (**30**) recorded from solutions in H_2O–CH_3CN (A), and D_2O–CH_3CN (B), and hydrocortison-21-yl sulfate anion (**31**) recorded from solutions in H_2O–CH_3CN (C), and D_2O–CH_3CN (D); (collision energy 42 eV; cone voltage 46 V).

that CID spectra obtained from solutions of both **30** and **31** in D_2O show a signal at *m/z* 98, indicating that an exchangeable proton is transferred (Fig. 8B and 8D). These spectra also show a low intensity peak at *m/z* 97 in addition to that of the more intense *m/z* 98 ion, indicating that pathway a competes favorably with another mechanism (b) in which a carbon-bound

proton is transferred from the C-16 position. Moreover, the formation of the *m*/*z* 81 ion appears to follow a pathway similar to that illustrated in Scheme 11(a) since this signal was not affected by the deuterium exchange.

Based on the results of the current study, and on the information from previous investigations on the mass spectrometric fragmentation of sulfate anions,[12,14,15,17] the following generalizations can be made. (1) When protons are available at the C-2 position, and C-1 and C-2 are sp^3 hybridized and able to accommodate a double bond, a proton from the C-2 position is transferred to the sulfate moiety and the C–O bond is broken to form a bisulfate anion (*m*/*z* 97). (The CID MS of cortison-21-yl sulfate suggests that more distant protons can also be eliminated.) (2) Vinyl and phenyl sulfates do not undergo the aforementioned bisulfate elimination to form the *m*/*z* 97 ion. The spectra of these anions show a peak for the loss SO_3 and a peak at *m*/*z* 80 for the $SO_3^{-\bullet}$. (3) Benzylic and allylic sulfates, which can give rise to a resonance-stabilized radical by the homolytic fission of the C–O bond, yield $SO_4^{-\bullet}$ anion as the major fragment observed at *m*/*z* 96.

An understanding of these collisionally-induced dissociation patterns should contribute significantly to the recognition and characterization of organic sulfates.

Experimental

General procedures

All reactions were carried out under argon using dry and freshly distilled solvents unless otherwise noted. [2,2-$^2H_2$]-Hexadecanoic acid was purchased from Cambridge Isotopes. Sodium dodecyl sulfate, hexadecan-1-ol, 2-methylpentan-2-ol, 3-methylpentan-3-ol, (−)-isopinocamphol, 1-adamantol, dehydroepiandrosteronyl sulfate, dihydrocholesterol, estrone, estradiol, (−)-linalool, phenol, 2-phenylethanol, and benzyl alcohol were Aldrich reagents. 5α-Androst-2-en-17-one, cortison-21-yl sulfate, and hydrocortison-21-yl sulfate were obtained from Steraloids Inc. (RI). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Unity 500 instrument operating at 499.93 MHz and a Varian XL 400 spectrometer operating at 100.58 MHz, using Me_4Si as the internal standard. Chemical shifts are reported in ppm and *J* values are given in Hz.

[1,1-$^2H_2$]Hexadecan-1-ol.[28] [1,1-$^2H_2$]Hexadecan-1-ol was prepared by a described procedure reducing methyl hexadecanoate with $LiAlD_4$. δ_C (100 MHz, $CDCl_3$) 14.1, 22.7, 25.7, 29.4, 29.4, 29.6, 29.6, 29.6, 29.7, 31.9, 32.6, 62.2 (quintet, *J* 20 Hz).

[2,2-$^2H_2$]Hexadecan-1-ol.[29] [2,2-$^2H_2$]Hexadecan-1-ol was prepared by a described procedure reducing [2,2-$^2H_2$]hexadecanoic acid with $LiAlH_4$. δ_H (500 MHz, $CDCl_3$) 0.88 (3 H, t, *J* 7.0 Hz), 1.26 (26 H, m) 3.64 (2 H, s). δ_C (100 MHz, $CDCl_3$) 14.1, 22.7, 25.5, 29.3, 29.4, 29.6, 29.7, 31.9, 31.9 (quintet, *J* 22 Hz), 62.9.

5α-[3α-$^2H_1$]Cholestan-3β-ol and 5α-[3β-$^2H_1$]cholestan-3α-ol. Treatment of cholestan-3-one with $LiAlD_4$ according to the procedure described above,[28] provided the title cholestanols as a mixture of two diastereomers (ratio 7 : 1, 67% overall yield), which was separated by flash chromatography (hexane–Et_2O, 7 : 3). The signal for the H-3 proton at δ_H 3.55 observed in the ^{1}H NMR spectrum of 5α-cholestan-3β-ol[30] was absent in that of the deuteriated β-epimer. Isomer **3β**: δ_C (100 MHz, $CDCl_3$) 12.0, 12.2, 18.3, 21.2, 22.5, 22.8, 23.8, 24.2, 28.0, 28.2, 28.7, 31.4, 32.0, 35.4, 35.5, 35.8, 36.1, 37.0, 38.1, 39.5, 40.0, 42.5, 44.8, 54.3, 56.2, 56.4, 70.8 (t, *J* 20 Hz). Similarly, in the ^{1}H NMR spectrum of the deuteriated β-epimer, a signal for an H-3 proton, observed at δ_H 4.0 (br s) for its non-deuteriated analog, was absent. Isomer **3α**: δ_C (100 MHz, $CDCl_3$) 11.2, 12.1, 18.7, 20.8, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.6, 28.9, 32.0, 32.1, 35.5, 35.8, 35.9, 36.1, 36.2, 39.1, 39.5, 40.0, 42.6, 54.3, 56.2, 56.5, 66.2 (t, *J* 20 Hz).

5α-[2,2,4,4-$^2H_4$]Cholestan-3β-ol and 5α-[2,2,4,4-$^2H_4$]cholestan-3α-ol. Both these compounds were prepared by the deuterium exchange of 5α-cholestan-3-one with Na–D_2O–dioxane,[31,32] followed by the reduction of the carbonyl group.[31] The diastereomeric mixture (ratio 10 : 1) of deuteriated cholestanols was separated by flash chromatography (hexane–Et_2O, 7 : 3). Isomer **3β**: δ_C (100 MHz, $CDCl_3$) 12.1, 12.3, 18.7, 21.2, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.7, 31.4 (m), 32.1, 35.4 (br s), 35.5, 35.8, 36.2, 36.8 (br s), 38.1 (m), 39.5, 40.0, 42.6, 44.8 (br s), 54.3, 56.3, 56.5, 71.1 (br s). Isomer **3α**: δ_C (100 MHz, $CDCl_3$) 11.2, 12.1, 18.7, 20.8, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.8 (m), 29.6 (br s), 32.0 (br s), 32.1, 35.5, 35.7, 35.8 (m), 36.0, 36.2, 39.0 (br s), 39.5, 40.0, 42.6, 54.3, 56.2, 56.5, 66.4 (br s).

5α-Cholestan-3α-ol. 5α-Cholestan-3α-ol was obtained as a minor stereoisomer by the reduction of cholestan-3-one with $LiAlH_4$ (ratio 8 : 1). The product was isolated by flash chromatography.

5α-Androst-2-en-17β-ol. Reduction of 5α-androst-2-en-17-one with $LiAlH_4$ provided the desired β-alcohol as a single diastereomer. δ_C (100 MHz, $CDCl_3$) 11.1, 11.7, 20.5, 23.4, 28.6, 30.3, 30.4, 31.4, 34.7, 35.6, 36.7, 39.8, 41.5, 42.9, 51.0, 54.2. 81.9, 125.8, 125.9.

5α-[17-$^2H_1$]Androst-2-en-17β-ol. Reduction of 5α-androst-2-en-17-one with $LiAlD_4$ yielded the product alcohol as a single diastereomer. The ^{13}C NMR spectrum of the product showed that the C-17 signal at δ_C 81.9 had changed to a triplet (t, *J* 21 Hz).

5α-[16,16-$^2H_2$]Androst-2-en-17β-ol. Following the procedure described above, the title dideuteriated alcohol was obtained starting from 5α-androst-2-en-17-one. An examination of the ^{13}C NMR spectrum of the product showed the absence of a C-16 signal, which is observed at δ_C 30.4 in that of its non-deuteriated analog.

Sulfation of alcohols

Sulfates of primary and secondary alcohols were prepared using DMF–SO_3 complex (1.1 eq.) in DMF in the presence of pyridine at 40 °C for 1 h.[22,33] Tertiary alcohols were sulfated using pyridine–SO_3 complex (2 eq.) in CCl_4 at 0 °C for 16 h.[15]

Mass spectrometry

The CID mass spectra were recorded using a Micromass (Beverly, MA) Quattro I triple quadrupole mass spectrometer equipped with an electrospray ion source. The source temperature was held at 85 °C. The argon gas pressure in the collision cell was adjusted to attenuate precursor ion transmission by 50%. Collision energy was optimized for each experiment and laboratory frame values are given in each corresponding figure caption. Samples were infused into the ESI source as acetonitrile–water solutions at a rate of 5 μl min^{-1}. To compare the relative intensities of product ions derived from 3α and 3β epimers of 5α-cholestan-3-yl sulfate, 5α-[3-^{2}H]cholestan-3-yl sulfate and 5α-[2,2,4,4-$^2H_4$]cholestan-3-yl sulfate, CID spectra of parent anions were recorded under identical conditions (laboratory frame of reference collision energy: 40.0 eV). For each recording 10 acquisition scans were co-added by multiple-channel analysis (MCA). After recording three profiles in this way, the sample was changed to its epimer and three profiles were recorded. The overall experiment was repeated immediately thereafter by switching samples. Thus, for each set of 3α and 3β epimers, twelve repetitive CID experiments were

conducted. For deuterium exchange experiments, **30** and **31** were dissolved in D_2O and investigated by negative-ion ESI.

Acknowledgements

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References

1 (*a*) X. Fu, M. L. G Ferreira, F. J. Schmitz and M. Kelly, *J. Org. Chem.*, 1999, **64**, 6706; (*b*) J. A. Kalaitzis, P. de A. Leone, L. Harris, M. S. Butler, A. Ngo, J. N. A. Hooper and R. J. Quinn, *J. Org. Chem.*, 1999, **64**, 5571; (*c*) T. Wakimoto, A. Maruyama, S. Matsunaga and N. Fusetani, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 727; (*d*) F. D. Horgan, B. Sakamoto and P. J. Scheuer. *J. Nat. Prod.*, 2000, **63**, 210; (*e*) Y. Nakao, S. Matsunaga and N. Fusetani, *Tetrahedron Lett.*, 1993, **34**, 1511.

2 Y. Kikuchi, M. Ishibashi, T. Sasaki and J. Kobayashi, *Tetrahedron Lett.*, 1991, **32**, 797.

3 M. N. Rao, A. E. Shinnar, L. A. Noecker, T. L. Chao, B. Feibush, B. Snyder, I. Sharkansky, A. Sarkahian, X. Zhang, S. R. Jones, W. A. Kinney and M. Zasloff, *J. Nat. Prod.*, 2000, **63**, 631.

4 G. F. Pauli, U. Matthiesen and F. R. Fronczek, *Phytochemistry*, 1999, **52**, 1075.

5 K. Hostettmann and A. Marston, *Chemistry and Pharmacology of Natural Products. Saponins*, Cambridge University Press, Cambridge, 1995.

6 (*a*) C. N. Falany, *FASEB J.*, 1997, **11**, 1; (*b*) R. M. Weinshilboum, D. M. Otterness, I. A. Aksoy, T. C. Wood, C. Her and R. B. Raftogianis, *FASEB J.*, 1997, **11**, 3; (*c*) C. N. Falany, *FASEB J.*, 1997, **11**, 206; (*d*) K. G. Bowman and C. R. Bertozzi, *Chem. Biol.*, 1999, **6**, R9; (*e*) J. W. Kehoe and C. R. Bertozzi, *Chem. Biol.*, 2000, **7**, R57.

7 C. D. Klaassen and J. W. Boles. *FASEB J.*, 1997, **11**, 404.

8 (*a*) E. Baumann, *Ber. Dtsch. Chem. Ges.*, 1876, 54; (*b*) W. B. Jakoby, R. D. Sekura, E. S. Lyon, C. J. Marcus and J. L. Wang, in *Enzymatic Basis of Detoxification*, ed. W. B. Jacoby, Academic Press, New York, 1980, Vol II, p. 199; (*c*) G. J. Mulder and W. B. Jakoby, in *Conjugation Reactions in Drug Metabolism: An Integrated Approach*, ed. G. J. Mulder, Taylor and Francis, Philadelphia, 1990, p. 107.

9 (*a*) C. C. Lai, J. A. Miller, E. C. Miller and A. Liem, *Carcinogenesis*, 1985, **6**, 1037; (*b*) H. Glatt, *FASEB J.*, 1997, **11**, 314; (*c*) A. E. Buhl, D. J. Waldon, C. A. Baker and G. A. Johnson, *J. Invest. Dermatol.*, 1990, **95**, 553.

10 K. G. Bowman, S. Hemmerich, S. Bhakta, M. S. Singer, A. Bistrup, S. D. Rosen and C. R. Bertozzi, *Chem. Biol.*, 1998, **5**, 447.

11 J. McCormick, Y. Li, K. McCormick, H. I. Duynstee, A. K. van Engen, G. A. van der Marel, B. Ganem, J. H. van Boon and J. Meinwald, *J. Am. Chem. Soc.*, 1999, **121**, 5661.

12 (*a*) L. O. G. Weidolf, E. D. Lee and J. D. Henion, *Biomed. Environ. Mass Spectrom.*, 1988, **15**, 283; (*b*) H. Zhang and J. Henion, *Anal. Chem.*, 1999, **71**, 3955.

13 (*a*) K. A. Thomsson, N. G. Karlsson and G. C. Hansson, *J. Chromatogr.*, *A*, 1999, **854**, 131; (*b*) A. Tjernberg, P. O. Edlund and B. Noren, *J. Chromatogr.*, *B*, 1998, **715**, 395; (*c*) S. Ikegawa, T. Yanagihara, N. Murao, H. Watanabe, J. Goto and T. Niwa, *J. Mass Spectrom.*, 1997, **32**, 401; (*d*) B. L. Ackermann, T. A. Gillespie, B. T. Regg, K. F. Austin and J. E. Coutant, *J. Mass Spectrom.*, 1996, **31**, 681.

14 S. Murray, N. B. Rendell and G. W. Taylor, *J. Chromatogr.*, 1996, **738**, 191.

15 B. Boss, E. Richling, M. Herderich and P. Schreier, *Phytochemistry*, 1999, **50**, 219.

16 R. W. Reiser and A. J. Fogiel, *Rapid Commun. Mass Spectrom.*, 1994, **8**, 252.

17 P. Rudewicz and K. M. Straub, *Anal. Chem.*, 1986, **58**, 2928.

18 K. B. Tomer and M. L. Gross, *Biomed. Environ. Mass Spectrom.*, 1988, **15**, 89.

19 S. J. Gaskell and M. H. Reilly, *Rapid Commun. Mass Spectrom.*, 1988, **2**, 139.

20 J. March, *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, Wiley, New York, 1992, p. 896; several examples of base-catalyzed β-elimination of sulfates have been described (J. H. Thomas and N. Tudball, *Biochem. J.*, 1970, **119**, 57P; J. H. Thomas, K. S. Dogson and N. Tudball, *Biochem. J.*, 1968, **110**, 687; R. Contestabile, T. Jenn, M. Akhtar, D. Gani and R. A. John, *Biochemistry*, 2000, **39**, 3091; H. Ueno, J. J. Likos and D. E. Metzler, *Biochemistry*, 1982, **18**, 4387; R. A. John and P. Fasella, *Biochemistry*, 1969, **11**, 4477; L. J. Fowler and R. A. John, *Biochem. J.*, 1972, **130**, 569; A. G. Brown, D. F. Corbett, A. J. Eglington and T. T. Howarth, *Tetrahedron*, 1983, **39**, 2551). To the best of our knowledge there are no reports of the gas phase cyclic mechanism that we propose here for the elimination of bisulfate. However, analogous oxy-Cope rearrangements of deprotonated diallyl ethers have been studied by Bowie and co-workers: P. C. H. Eichinger and J. H. Bowie, *J. Chem. Soc.*, *Perkin Trans. 2*, 1987, 1499; P. C. H. Eichinger, S. Dua and J. H. Bowie, *Int. J. Mass Spectrom. Ion Processes*, 1994, **133**, 1.

21 (*a*) J. Bredt, H. Thouet and J. Schmitz, *Liebigs Ann. Chem.*, 1924, 437; (*b*) P. M. Warner, *Chem. Rev.*, 1989, **89**, 1067; (*c*) W. Maier and P. v. R. Schleyer, *J. Am. Chem. Soc.*, 1981, **103**, 1891.

22 K. Chatman, T. Hollenbeck, L. Hagey, M. Vallee, R. Purdy, F. Weiss and G. Siuzdak, *Anal. Chem.*, 1999, **71**, 2358.

23 S. J. Gaskell, *Biomed. Environ. Mass Spectrom.*, 1988, **15**, 99.

24 J. Yinon, L. D. Betowski and R. D. Voyksner, in *Applications of LC-MS in Environmental Chemistry*, ed. D. Barceló, Elsevier, Amsterdam, 1996, p. 187.

25 G. K. Poon, G. M. F. Bisset and P. Mistry, *J. Am. Soc. Mass Spectrom.*, 1993, **4**, 588.

26 P. A. Wabnitz, J. H. Bowie and M. J. Tyler, *Rapid Commun. Mass. Spectrom.*, 1999, **13**, 2498.

27 C. A. Grob and P. W. Schiess, *Angew. Chem.*, *Int. Ed. Engl.*, 1967, **6**, 1.

28 (*a*) H. Taguchi, B. Paal and W. L. F. Armarego, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, **3**, 303; (*b*) A. Firouzi, F. Atef, A. G. Oertli, G. D. Stucky and B. F. Chmelka, *J. Am. Chem. Soc.*, 1997, **119**, 3596.

29 L. Debrauwer, A. Paris, D. Rao, F. Fournier and J. C. Tabet, *Org. Mass Spectrom.*, 1992, **27**, 709.

30 C. J. Pouchert, *Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*, Aldrich Chemical Company, Milwaukee, 1993, vol. 3, 566C, 567A.

31 J. Karliner, H. Budzikiewicz and C. Djerassi, *J. Org. Chem.*, 1966, **31**, 710.

32 R. C. Cookson, D. P. G. Hamon and R. E. Parker, *J. Chem. Soc.*, 1962, 5014.

33 S. Futaki, T. Taike, T. Yagami, T. Ogawa, T. Akita and T. Kitagawa, *J. Chem. Soc.*, *Perkin Trans. 1*, 1990, 1739.