CHEMICAL AND SPECTROSCOPIC CHARACTERIZATION OF MONOBACTAMS

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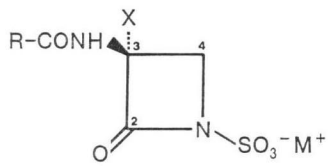
A set of chemical and spectroscopic studies using IR and FAB-mass spectral data has been developed to identify monobactams thereby differentiating them from other families of β-lactam antibiotics.

A rapid and simple set of experiments has been developed to identify monobactams in aqueous media. These experiments allow the differentiation of monobactams from other β-lactam antibiotics by studying the chemistry of the $-SO_3^-$ group, which is attached to the nitrogen of the β-lactam nucleus and uniquely distinguishes the monobactam structure. Monobactams are monocyclic β-lactam antibiotics that are characterized by the 2-oxoazetidine-1-sulfonic acid nucleus as shown in Fig. 1. Both methoxy ($X=OCH_3$) and demethoxy ($X=H$) monobactams are produced by bacteria.[1] We have recently isolated several demethoxy monobactams possessing oligopeptide side chains. The isolation of two of these monobactams, namely SQ 28,502 and SQ 28,503 was described previously.[2] These compounds are the largest monobactams reported to date, and have molecular weights of 1,462 and 1,446, respectively. During the initial characterization of both SQ 28,502 and SQ 28,503, it was not readily apparent that these compounds were monobactams. Although the presence of a β-lactam was detected (IR $\nu_{C=O}$, 1760 cm^{-1}), the identification of the monobactam nucleus still remained obscure due to the size and complexity of these molecules. For example, neither SQ 28,502 nor SQ 28,503 possess the typical electrophoretic mobility at low pH associated with several reported monobactams.[3,4] Furthermore, the ^1H NMR signals assigned to the H-3 and H-4 protons of the β-lactam that are characteristic of demethoxy monobactams,[4] were not clearly evident.[2] Since the electrophoretic behavior and the ^1H NMR data were the major tools previously used for the identification of monobactams,[4] it became necessary to develop a method that would allow us to unequivocally identify the 2-oxoazetidine-1-sulfonic acid nucleus. This has been accomplished by the following spectroscopic and chemical studies: (1) examination of the IR spectra of monobactam salts in H_2O, (2) identification of the $N-SO_3^-$ group in the monobactam by treatment with $BaCl_2/HNO_2$, (3) analysis of fast atom bombardment (FAB)-mass spectral fragmentation of monobactams. Details of these three methods are presented herein, together with their application to the study of complex β-lactam compounds, SQ 28,502 and SQ 28,503.

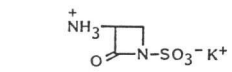
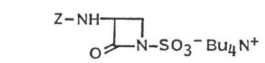
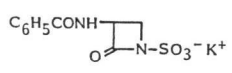
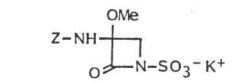
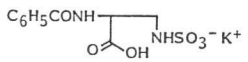
Fig. 1. Structure of the 2-oxoazetidine-1-sulfonic acid nucleus.



X = OCH_3
X = H
R = Acyl or peptide

The IR spectra were recorded in H_2O using Irtran cells (Barnes Analytical). This method

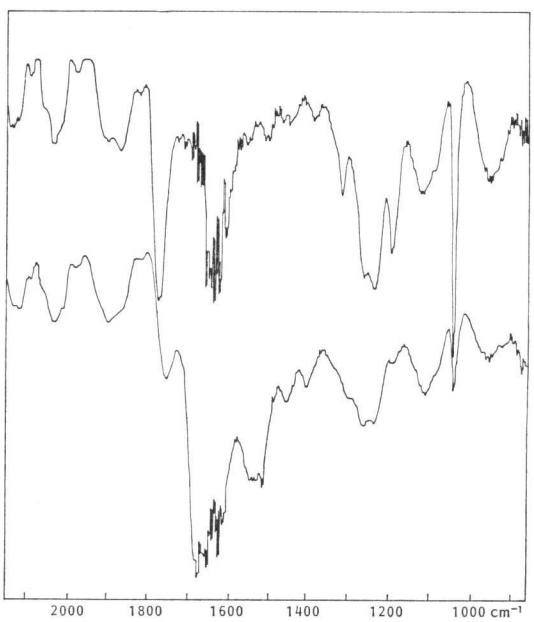
Table 1. IR data for monobactams and related compounds.

No.	Compounds	$\nu_{C=O}$ (β-Lactam)	$\nu_{S=O}$ Stretching frequencies (cm^{-1})			
1	$CH_3(CH_2)_6SO_3^-Na^+$				1170	1040
2	$C_6H_{11}NHCH_2CH_2SO_3^-Na^+$			1205 sh	1170 s	1040
3	$C_{12}H_{25}OSO_3^-Na^+$			1205	1170 sh	1050
4	$NH_2(COOH)CHCH_2SSO_3^-Na^+$		1240 sh	1200	1103	1028
5	$NH_2SO_3^-Na^+$		1235		1180	1045
6	$C_6H_{11}NHSO_3^-Na^+$		1240	1205	1175 s	1035
7	$C_6H_5CH_2CONHSO_3^-Na^+$		1220 br		1190	1040
8		1779	1328, 1271, 1248, 1204		1116 br	1050
9		1758	1344, 1275→1185		1110 br	1047
10		1760	1320, 1260→1220 br			1040
11		1775	1339, 1268→1204		1115	1046
12	SQ 28,502	1759	1330, 1268→1199		1115	1046
13	SQ 28,503	1757	1328, 1257→1195		1110	1046
14			1220 br		1170	1040

s=Strong, sh=shoulder, br=broad. IR spectra were taken on a Perkin-Elmer 983 infra-red spectrophotometer (3600 data station) using Irtran-2-ZnS (0.2 mm thickness) cells; a path length of 0.015 mm; *ca.* 1 mg/25 μl H_2O was used.

eliminates the undesired solid state effects that are associated with KBr pellets,[5] and permitted the examination of the clearly resolved SO stretching vibrations in the 1300~1100 cm^{-1} region, together with the β-lactam carbonyl absorptions. In Table 1, data is presented on the β-lactam carbonyl and SO stretching frequencies for monobactams, and for model compounds, all of which contain an $-SO_3^-$ group. Clear differences for the SO stretching vibrations are observed in the open chain compounds (**1**~**7**, Table 1), when the $-SO_3^-$ group is attached to one of the following; carbon (1170 cm^{-1}, **1** and **2**), oxygen (1205 cm^{-1}, **3**), sulfur (1200 cm^{-1}, **4**) or nitrogen (1240 cm^{-1}, **5**~**7**). These results are in agreement with published data,[6] and were used as a first step in establishing the point of attachment of the $-SO_3^-$ group to nitrogen in the monobactam structures. All compounds under study containing an $N-SO_3^-$ group (**5**~**14**)

Fig. 2. IR spectra in H_2O of 3-aminomonobactamic acid (**8**) and SQ 28,502 (**12**) in the region of 2000 to 900 cm^{-1}.



show SO stretching frequencies in the region of 1300~1200 cm^{-1}. The open chain compounds (**5**~**7**) exhibit a broad band in this region, whereas the monobactams (**8**~**13**) exhibit a set of four closely spaced bands in the same region. IR spectra of a simple monobactam, 3-aminomonobactamic acid (**8**) and of a complex monobactam (**12**) are given in Fig. 2. Both compounds show SO stretching vibrations at 1328, 1271, 1248 and 1204 cm^{-1} and a broad band at 1115 cm^{-1}. The four bands in the 1300~1200 cm^{-1} region are observed in the IR spectra of both methoxy and demethoxy monobactams, and appear to be characteristic of the 2-oxoazetidine-1-sulfonic acid moiety. In order to examine the effect of the β-lactam ring on the SO stretching vibrations, monobactam **10** was hydrolyzed using 1 N NaOH to give compound **14**, in which the β-lactam ring has been opened to give an alkyl sulfamic acid ($RNHSO_3^-$, **14**). The IR data show that the four bands associated with **10** (and other monobactams) are no longer resolved in compound **14**. The band at 1328 cm^{-1} disappears and a broad envelope centering around 1220 cm^{-1} is observed. As expected, the IR spectrum of **14** has the features of compounds **6** and **7** in the region of the SO stretching bands, since all three compounds are open chain derivatives possessing an $NHSO_3^-$ functionality. In conclusion, four closely spaced SO stretching bands in the region of 1300~1200 cm^{-1}, in conjunction with β-lactam carbonyl absorptions (at *ca.* 1760 cm^{-1}) can be used as one identification tool for monobactams.

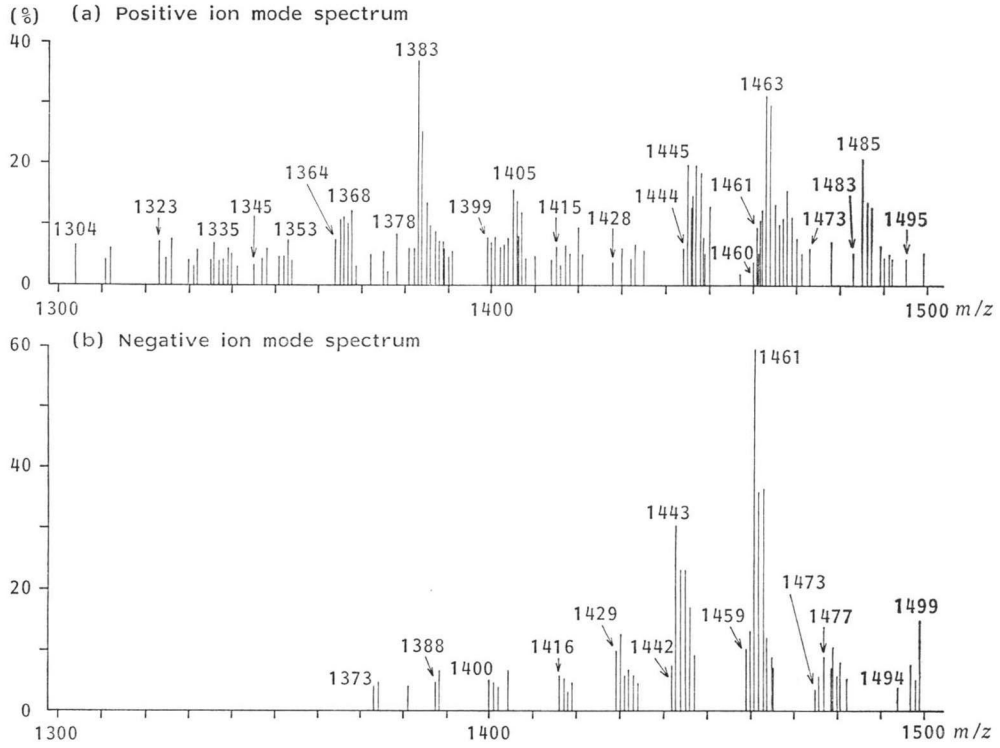
The second supportive evidence for the presence of a monobactam is chemical detection of a sulfamic acid group. The sulfamic acid ($RNHSO_3^-$) group is detected by treatment with $BaCl_2$/2 N HCl followed by the addition of $NaNO_2$.[7] The nitrous acid formed *in situ* causes release of sulfate, and precipitation of $BaSO_4$ is observed. It is generally believed that intermediates of the type RN(NO)-SO_3H are generated,[8] where the NO function replaces the proton attached to the N–SO_3 group. These intermediates readily decompose, causing release of sulfate. Hence, the reaction with primary or secondary sulfamic acids proceeds readily with $BaCl_2/HNO_2$.[9] However, monobactams may be considered as tertiary sulfamic acids [R(CO)NSO_3H]. Since there is no proton attachment to the N–SO_3 group the reaction conditions as described above are not in all cases sufficient to cause release of sulfate. Therefore, the monobactam has to be converted firstly to a seondary sulfamic acid. This step is achieved with a mild base treatment (1 N NaOH, 60°C, 15 minutes). The β-lactam ring is opened to the secondary sulfamic acid; subsequent addition of $BaCl_2$/2 N HCl followed by $NaNO_2$ now gives the expected $BaSO_4$ precipitate.

Methoxy and demethoxy monobactams exhibit differing degrees of lability toward acid. Consequently the examination of the different conditions for efficient release of sulfate can be a means of distinguishing methoxy from demethoxy monobactams. Methoxy monobactams (*e.g.* SQ 26,180[3] and **11**) are acid labile. Thus, the β-lactam is opened rapidly by 2 N HCl at room temperature and addition of $BaCl_2$ followed by $NaNO_2$ results in precipitation of $BaSO_4$. Demethoxy monobactams (*e.g.*, **8**~**10**, **12** and **13**) are more resistant to acid hydrolysis. Only after prolonged heating (2 N HCl, 60°C, 5 hours) is the β-lactam ring opened; subsequent addition of $BaCl_2/NaNO_2$ results in $BaSO_4$ precipitation. It should be noted that no sulfate is released from SQ 28,502 or SQ 28,503 by acid alone prior to the addition of $NaNO_2$; therefore, the hydrolysis of an O–SO_3 group may be ruled out. These experiments were performed on microgram quantities of monobactam and visualization of $BaSO_4$ precipitation was possible for 0.1 mM solutions.

Monobactams containing O–SO_3 groups have been reported.[4] Although, in general, cleavage of the sulfamic group is expected to be more rapid than hydrolysis of an *O*-sulfate group,[10] the vigorous acid conditions used to hydrolyze the demethoxy monobactams, **8**~**10**, **12** and **13**, to the sulfamic acid

Fig. 3. Fast atom bombardment mass spectra (in glycerol) of SQ 28,502 in the positive ion mode (a) and the negative ion mode (b).

Spectra were recorded on a VG-ZAB-1F instrument (VG Analytical Ltd.).



could cause hydrolysis of an alkyl *O*-sulfate, if this group were present elsewhere in the molecule. Therefore, the total amount of hydrolyzable sulfate was calculated. This was accomplished in the following manner: the demethoxy monobactams **8**~**10**, **12** and **13** were hydrolyzed in 6 N HCl, 107°C, 15 hours and the total amount of hydrolyzable sulfate ion per monobactam was quantitated. Measurements were made by ion chromatography. These experiments showed that only one sulfate ion was released per molecule. Since the N–SO_3 group has been firmly established in the monobactams, these results preclude the presence of an O–SO_3 group in the molecule.

Further supporting evidence for the presence of a monobactam can be obtained by FAB-mass spectrometry.[11] The loss of sulfur trioxide has been observed from all monobactams analyzed to date,[12] and appears to be characteristic of a 2-oxoazetidine-1-sulfonic acid compound. For example, FAB-mass spectral data for aztreonam,[13] a synthetic monobactam antibiotic has recently been reported.[14] It was shown that the principle high mass fragment ion in the positive mass spectrum results from the loss of SO_3 from the $(M+H)^+$ ion.

An example of the FAB-mass spectrum of a monobactam is shown in Fig. 3, for SQ 28,502 (MW 1,462). Inspection of the positive ion FAB-mass spectrum (Fig. 3a) revealed the protonated parent ion (*m/z* 1,463) and the fragment ion (*m/z* 1,383) corresponding to the loss of SO_3. This cleavage was confirmed by a mass-analyzed ion kinetic energy (MIKE) spectrum[15] in the positive ion mode. In Fig. 3b, we recorded the negative ion spectrum of SQ 28,502. The $(M-H)^-$ ion, *m/z* 1,461, is present, but loss of SO_3 is not observed in this mode.

In conclusion, the sensitive chemical and spectroscopic tests described above can be applied to small quantities of monobactams and allow their distinction from all other families of β-lactam antibiotics. Application of these methods to SQ 28,502 and SQ 28,503, both of which contain complex oligopeptide side chains, enabled us to establish the presence of a 2-oxoazetidine-1-sulfonic acid nucleus in these β-lactam antibiotics.

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