Sensitized Photolysis of Predioxin Anion in Methanol in the Presence of Excess Triethylamine. A 10.0-mL methanolic solution of predioxin (0.0496 g, 0.10 mmol), *m*-methoxyacetophenone (0.122 g, 0.81 mmol), and dodecane (26.2 mg, 0.154 mmol) was prepared. To two 4.0-mL samples of the solution was added 1 equiv of triethylamine in order to convert predioxin to its anion. Then, to each sample was added 16.0 mg (0.16 mmol) of triethylamine. The mole ratio of predioxin anion to triethylamine was 1:4. The samples were degassed and irradiated according to the general procedure. Quantum yields were measured using cyclopentanone actinometry. A 10.0-mL methanolic solution of cyclopentanone (2.121 g, 25.2 mmol) and dodecane (0.138 g, 0.809 mmol) was prepared. Two 4.0-mL samples were degassed and irradiated simultaneously with the predioxin samples.

Direct Photolysis of Predioxin Anion in Absolute Methanol. A 10.0-mL solution of predioxin (0.0495 g, 0.998 mmol) and dodecane (0.0749 g, 0.4397 mmol) was prepared in methanol, providing a 0.01 M solution of predioxin. Two 4.0-mL samples were placed in two separate quartz tubes, and to each a micro drop of phenolphthalein indicator was added and the solution neutralized with methoxide (1.0 M). The solutions were then degassed, sealed in vacuum, and irradiated for 3 h. Quantum yields were determined using cyclopentanone actinometry; two 4.0-mL samples of 2.0 M cyclopentanone in absolute methanol were used.

Photolysis of Predioxin Anion in Solvents of Different Polarity in the Presence of Excess Triethylamine. Stock solutions (10.0 mL) containing 0.01 M predioxin were prepared in absolute methanol, dibutyl ether (distilled from calcium hydride just before use), and H_2O/CH_3CN (70:30). Two 4.0-mL samples of each of the stock solutions were transferred into separate quartz tubes and 1 microdrop of phenolphthalein indicator was added to each tube. Triethylamine was added drop by drop with a micro syringe until neutralization was achieved. At this point triethylamine (0.041 g, 0.40 mmol) and dodecane (50 μL, 0.22 mmol) were added, and the samples were degassed, sealed in vacuum, and irradiated for 3 h. Cyclopentanone actinometry was employed, using 2.0 M solutions of cyclopentanone in dibutyl ether to measure quantum yields in the dibutyl ether runs and 2.0 M solutions of cyclopentanone in methanol to measure quantum yields in the methanol and H_2O/CH_3CN runs. Methanol solvent was used in the latter two solvent systems due to a solubility problem with cyclopentanone in H_2O/CH_3CN; however, the refractive index for these two solvent systems is very close (methanol n^{20}_D 1.329, H_2O/CH_3CN n^{20}_D 1.336).

Acknowledgment. We express our appreciation to the National Institute of Environmental Health Sciences (ES0040) for the support of this work.

Registry No. **1a**, 35245-80-8; **1a**·Na, 104156-80-1; **4b**, 3268-87-9; **8a**, 104156-81-2; **9a**, 104156-82-3; **10**, 104156-83-4; **11a**, 35822-46-9; **11b**, 58200-70-7; 3'-$H_3COC_6H_4COCH_3$, 586-37-8; 2,3,4,5-tetrachlorophenol, 4901-51-3; pentachlorophenol, 87-86-5.

Novel Potentiators of β-Lactam Antibiotics. Structures of SQ 28504 and SQ 28546

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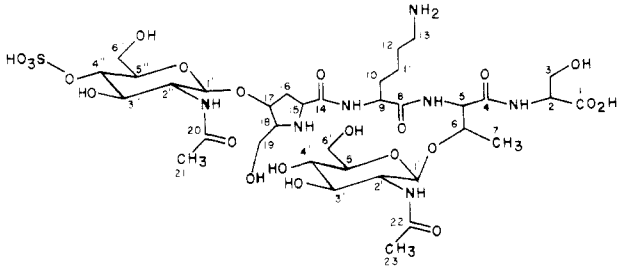
Received April 18, 1986

The structures of two novel potentiators of β-lactam antibiotics, namely, SQ 28504 (**1**) and SQ 28546 (**2**), are presented. The structure elucidations of these O-sulfated glycopeptides are based on NMR and FAB mass spectral data. Thermospray mass and MS/MS spectra were obtained of hydrolysates formed by treatment with carboxypeptidases and aided in the deduction of structures **1** and **2**.

The isolation and biological properties of two novel O-sulfated glycopeptides SQ 28504 (**1**) and SQ 28546 (**2**) have been reported.[1] These bacterial metabolites are found in the culture broth of *Chromobacterium violaceum*



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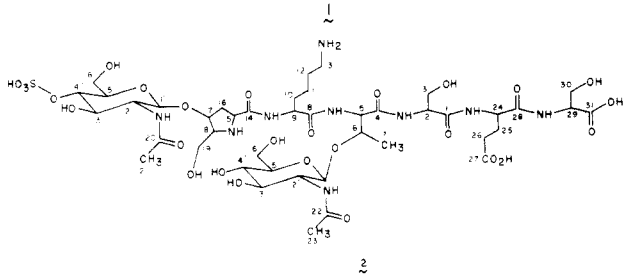

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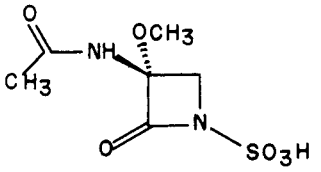

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and are coproduced with the monobactam[2] SQ 26180 (**3**). SQ 28504 and SQ 28546 potentiate the activity of β-lactam antibiotics against gram-negative organisms, inducing morphological changes. Bulge formation in the elongated cells is observed and culture growth is inhibited. These changes are similar to those reported for the bulgecins,[3,4] O-sulfated glycopeptides coproduced with the monobactam sulfacezin.[5] In the present paper, the structure elucidation of SQ 28504 and SQ 28546 based upon NMR and mass spectral data is discussed in detail. The unique assembly

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of the component parts and the amino acid sequence were determined by spectroscopy and hydrolysis reactions in combination with accurate mass measurements and MS/MS analysis of parent → daughter relationships.

Experimental Section

Nuclear Magnetic Resonance. ^1H and ^{13}C NMR spectra were acquired in D_2O on a JOEL XL-400 instrument. Dioxane served as internal standard (67.6 ppm) for ^{13}C spectra while the HDO resonance (4.73 ppm) was used for ^1H spectra.

Mass Spectrometry. Fast atom bombardment (FAB) mass spectra[6] were acquired on a VG-ZAB-2F mass spectrometer. The glycopeptide was dissolved in either thioglycerol or a dithiothreitol–dithioerythritol mixture and sputtered using 8-KeV Xe atoms. Positive and negative low resolution mass spectra were obtained at 1:1500 resolution while accurate mass measurements were made at 1:8000 resolution by peak matching. For accurate mass measurements polyethylene glycols and Ethaquad were employed as ca. 1% aqueous internal standards.[7,8] MS/MS spectra[9] were acquired in the following manner. Secondary ions were accelerated to 8-KeV energy, mass analyzed, and subjected to collision (50% main beam reduction; N_2), and the resulting fragment ions were determined by scanning the electric sector of the reversed-geometry VG-ZAB-2F. Daughter ion resolution is approximately 100–200 and is sufficient to define fragment masses from peak centroids.

Thermospray mass and MS/MS spectra of amino acids formed by treatment of **2** with carboxypeptidases[10] were acquired on a Finnigan TSQ-4600 mass spectrometer and Vestec thermospray source. Twenty microliters of the resulting digest was injected onto a Whatman C18 column using a 60:40 water (0.1 M ammonium acetate)/acetonitrile mobile phase. MS/MS spectra were obtained by mass analyzing the expected protonated molecule in the first quadrupole, activating this species by collision (30 eV; 1 mtorr Ar) in the second quadrupole, and scanning the third quadrupole for fragment ions.

Results and Discussion

SQ 28504 (**1**) and SQ 28546 (**2**) are water soluble, amphoteric compounds. The molecular formulae were determined on the basis of microanalysis and FAB mass spectroscopy. Compound **1** has a molecular weight of 963 ($C_{35}H_{61}N_7O_{22}S$) and **2** has a molecular weight of 1179 ($C_{43}H_{73}N_9O_{27}S$). The two compounds show end absorption in the UV spectra. The IR spectra indicate the presence of OH and NH groups (3300 cm^{-1}), amide groups (1650 cm^{-1}), and sulfate (1250, 1050, and 650 cm^{-1}).

Mild acid hydrolysis (2 N HCl/aqueous MeOH) of each glycopeptide yields D-glucosamine. Acid hydrolysis of **1** in 6 N HCl (105 °C, 17 h) yields lysine, L-serine, and L-threonine in a 1:1:1 ratio,[1] together with an unusual amino acid, identified as 4-hydroxy-5-(hydroxymethyl)proline, by direct comparison with an authentic sample (obtained from acid hydrolysis of bulgecin A[11a]). This novel amino acid has been named bulgecinine and was recently synthesized by Wakamiya et al.[11b] Sulfate ion is also detected in the acid hydrolysate. Hydrolysis of **2** in 6 N HCl yields lysine, L-serine, L-threonine, and L-glutamic acid in a 1:2:1:1 ratio,[1] together with 4-hydroxy-5-(hydroxymethyl)proline and sulfate ion.

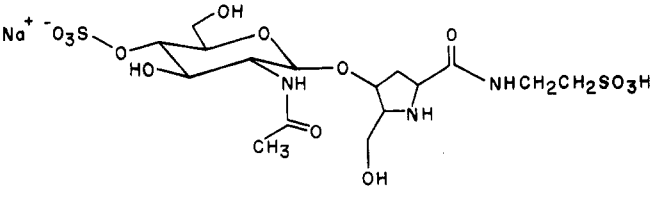
Table I. ^1H NMR Spectral Data for SQ 28504 (1)

position (protons)	δ(J, Hz)[a]
7 (3 H)	1.10 (d, J = 6.1)
11 (2 H)	1.26 (m)
12 (2 H)	1.46 (m)
10 (2 H)	1.64 and 1.78 (dd, J = 5 and 16)
21 and 23 (6 H)	1.84 (s)
16A (1 H)	2.08 (J_{AB} = 14, $J_{A,15}$ = 6, $J_{A,17}$ = 4)
16B (1 H)	2.52 (J_{AB} = 14, $J_{B,15}$ = 8, $J_{B,17}$ = 6)
13 (2 H)	3.15 (m)
18 (1 H)	3.40 (m)
19A (1 H)	3.45 (m, J_{gem} = 14.0)
19B (1 H)	3.74 (m, J_{gem} = 14.0)
2′ (1 H)	3.55 (m)
2″ (1 H)	3.65 (m)
15 (1 H)	3.95 (dd, 4, 6)
9 (1 H)	4.08 (m)
6 (1 H)	4.20 (m)
17 (1 H)	4.22 (m)
5 (1 H)	4.39 (d, 4.6)
1′ (1 H)	4.50 (d, 7.9)
1″ (1 H)	4.55 (d, 8.6)

[a] Serine protons not resolved between δ 3.5 and 3.8.

The ^1H NMR spectra of both **1** and **2** reveal the presence of two *N*-acetyl methyl groups (δ 1.84, s, 6 H) and a methyl group of threonine (δ 1.10, d, 3 H, J = 6 Hz) coupled to a methine proton (δ 4.22, m, 1 H, J = 6 Hz). There are signals for two anomeric protons in each molecule (δ 4.55, d, 1 H, J = 8.6 Hz and δ 4.50, d, 1 H, J = 7.9 Hz), indicating the presence of two sugars in both **1** and **2**. Proton spin-decoupling studies aided the analysis. The data for **1** are presented in Table I.

The data reveal the presence of threonine, lysine, and serine together with the partial coupling networks of 4-hydroxy-5-(hydroxymethyl)proline (bulgecinine) shown in Tables I and II. The chemical shifts and coupling constants assigned to 4-hydroxy-5-(hydroxymethyl)proline in **1** are compared to those found by Shinagawa et al.[4] in bulgecin and closely agree. In the region of δ 3–4 the protons were not all clearly resolved in neutral solution. The ^1H NMR spectrum was taken in acidic solution and some differences in chemical shifts were observed. These slight shifts enabled us to measure the coupling constants between the H-15, H-16, H-17, and H-18 protons; details are given in Table II. A comparison of the chemical shifts and coupling constants obtained for the 4-hydroxy-5-(hydroxymethyl)proline in compounds **1**, **2**, and **4** lead us to believe that the stereochemistry of this cyclic imino acid is the same in all three compounds.



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^1H NMR spectra of **1** obtained in neutral and acidic solution were compared. Differences in chemical shifts were observed for the δ-CH_2 protons of lysine and the 4-hydroxy-5-(hydroxymethyl)proline protons on carbons adjacent to nitrogen. This information revealed that the two H-13, the H-15, and H-18 protons are shifted due to their proximity to an unsubstituted nitrogen. This suggested that (1) lysine is linked through the α-CHNH–, (2) there are free amines on lysine and 4-hydroxy-5-(hydroxymethyl)proline, and (3) sulfate is attached in **1** (and **2**) through oxygen and not nitrogen.

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Table II. Comparison of ^1H NMR Spectral Data of SQ 28504 (1) with Bulgecin

position	δ (J, Hz) 1	bulgecin[4]
H_{15}	3.95 (dd 4,6)	4.21 (dd, 9, 6.6)
H_{16A}	2.08, (ddd, 14, 6, 4)	2.16 (ddd, 13.9, 6.6, 5)
H_{16B}	2.52 (ddd 14, 8, 6)	2.66 (ddd, 13.9, 9, 5.8)
H_{17}	4.22 (m, $J_{17,18}$ = 5)	4.39 (ddd, 5.8, 5.1, 4.2)
H_{18}	3.40 (m, $J_{17,18}$ = 5)	3.76 (m, 6.5, 6.0, 4.2)
H_{19A}	3.45 (m, J_{gem} = 14)	3.75 (m, J_{gem} = 14, 6)
H_{19B}	3.74 (m, J_{gem} = 14)	3.90 (m, J_{gem} = 14, 6.5)

Table III.[a] ^{13}C NMR Spectral Data for 1, 2, and 4

position (carbons)	δ 1	2	4
2	56.7	58.8 (CH)	
3[b]	61.9	61.8 (CH_2)	
5	60.6	61.6 (CH)	
6	70.9	71.0 (CH)	
7	17.7	17.4 (CH_3)	
9	55.8	55.6 (CH)	
10	29.2	29.1 (CH_2)	
11	24.4	23.5 (CH_2)	
12	32.5	32.5 (CH_2)	
13	40.6	40.8 (CH_2)	
15	59.3	59.4 (CH)	59.5
16	37.3	37.1 (CH_2)	36.7
17	80.2	79.3 (CH)	79.1
18	66.1	66.1 (CH)	66.2
19	59.4	59.6 (CH_2)	59.0
21, 23	23.4	23.5 (CH_3)	23.1
24		54.8 (CH)	
25		34.5 (CH_2)	
26		28.8 (CH_2)	
29		56.8 (CH)	
30		61.2 (CH_2)	
1′, 1″	100.6, 101.4	100.3, 101.3 (CH)	101.1
2′, 2″	56.3, 56.4	56.2, 56.5 (CH)	56.0
3′, 3″	73.2, 74.6	73.0, 74.7 (CH)	73.1
4′, 4″	76.7, 77.9	76.7, 77.9 (CH)	77.5
5′, 5″	75.4, 76.1	75.5, 75.6 (CH)	75.2
6′, 6″[b]	2 × 61.6	62.0, 62.2 (CH_2)	61.3

[a] For 1 only two CO signals at 174.9 and 175.3 ppm were observed. For 2 nine CO signals at 168.4, 169.1, 170.6, 174.9, 175.3, 177.9, 178.8, and 181.5 ppm were observed. [b] May be interchanged.

The configurations of the glycosidic linkages are both in the β-form as deduced from the following. Firstly, the coupling constants ($J_{1',2'}$ = 8.6 Hz and $J_{1'',2''}$ = 7.9 Hz) between H-1′ and H-2′ and H-1″ and H-2″ indicate that the anomeric protons are axial. Secondly, the chemical shifts of the anomeric carbons from ^{13}C data, discussed below, are similar to those of a methyl β-glycoside.

The ^{13}C NMR data from fully proton-decoupled ^{13}C NMR spectra for 1 and 2 are presented in Table III. Assignments were made with the aid of the INEPT[12] technique. The ^{13}C NMR chemical shifts[4] for the O-sulfated *N*-acetylglucosamine-4-hydroxy-5-(hydroxymethyl)proline portion of bulgecin A are included in Table III. The chemical shifts for this portion of 1, 2, and 4 are in very close agreement, suggesting the location of the SO_3 group at the 4″-position of the sugar in 1 and 2. Further evidence to support this assignment is provided by FAB–mass spectral data discussed below. The spectra of both 1 and 2 exhibit two anomeric carbon signals at 101.4 ppm and 100.6 ppm and at 100.3 and 101.3 ppm, respectively.

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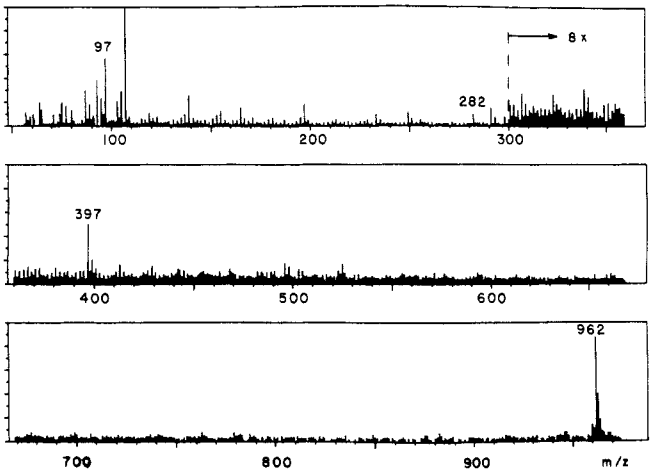

Figure 1. Negative FAB mass spectrum of SQ 28504 from a thioglycerol solution.

The glycosidic linkages are through oxygen of methine carbons based on chemical shift data. The signals assigned to C-6 and C-17 are shifted downfield by 6 and 9 ppm relative to resonances for hydroxyproline and threonine oxymethine carbons, respectively.[13] Since a glycosyl unit causes a downfield shift (7–10 ppm) at the carbon of attachment,[14] the glycosidic linkage in both 1 and 2 exist between the anomeric carbons and C-6 and C-17. Alternate hydroxymethyl sites of glycosidation (C-3, C-19, C-30, C-6′, and C-6″) were ruled out since no downfield shifts for these carbons were observed. The ^{13}C NMR data indicate that glycosidic linkages exist between each amino sugar and the peptide chain.

The ^{13}C NMR spectral data for 1 and 2 are similar. Only the extra carbon resonances assigned to serine and glutamic acid differentiate 1 and 2, suggesting that the two extra amino acids in 2 extend a linear peptide chain. Examination of the α-methine carbon chemical shift assignments aided the sequencing of amino acids. From NMR, and mass spectrometry data discussed below, the sequence, bulgecinine–lysine–threonine–serine was established in 1 and 2. In 2 this sequence is extended with glutamic acid and a terminal serine. The α-methine carbon of serine in 1 (56.7 ppm) is shifted 2 ppm upfield from the α-methine carbon of the serine in the peptide chain of 2 (58.8 ppm). Glutamic acid is therefore nonterminal and the α-methine carbon is assigned at 54.8 ppm. These assignments are made by comparison to literature data.[13-15] Confirmation of the proposed sequences in compounds 1 and 2 were obtained from mass spectral results discussed below.

Several ionization methods (field desorption, thermospray, electron and chemical ionization) were tried; however, only fast atom bombardment was capable of producing intact positive and negative parent ions of 1 and 2. The protonated and deprotonated molecule (Figure 1) of 1 were observed at *m/z* 964 and 962, respectively. The sodiated molecule was also evident at *m/z* 986. Similarly, 2 yielded positive and negative parents at *m/z* 1180 and 1178 as well as the sodiated parent at *m/z* 1202 (Figure 2). Accurate mass measurement of the $(M + H)^+$ ion of 2 yielded 1180.4432 consistent with a molecular formula of $C_{43}H_{74}N_9O_{27}S^+$ (calcd 1180.4414). Accurate mass measurement of the protonated molecule of 1 yielded 964.3698 (calcd 964.3668 for $C_{35}H_{62}N_7O_{22}S^+$). The former result is

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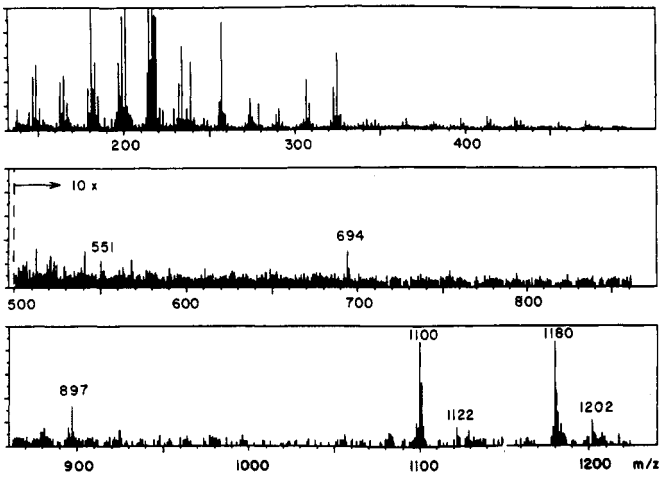


Figure 2. Positive FAB mass spectrum of SQ 28546 from a thioglycerol solution.

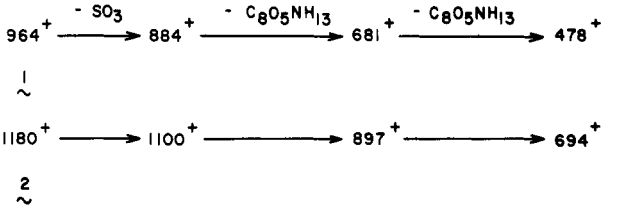
consistent with the microanalysis of the monopotassium salt of **2** [Found: C, 42.43; H, 6.02; N, 10.59; S, 2.57. Calcd: C, 42.40; H, 5.96; N, 10.35; S, 2.63].

Fragmentation was examined by MS/MS mapping of parent → daughter relationships as discussed in the Experimental Section. Compound **1** exhibited intense loss of 80 μm from the 964^+ parent in both its mass and MS/MS spectrum. Also evident in the MS/MS spectrum is loss of 203 μm (761^+) and subsequent 80 μm loss ($761^+ \rightarrow 681^+$). Loss of SO_3 accounts for the 80 μm loss, while loss of the sugar, *N*-acetylglucosamine, is responsible for the 761^+ fragment. An analogous sequence of fragments was observed from the MS/MS spectrum of the $(M + H)^+$ ion of **2**. Loss of SO_3 was seen ($1180^+ \rightarrow 1100^+$) as well as the loss of *N*-acetylglucosamine ($1100^+ \rightarrow 897^+$). The observation of a strong 97^- ion in the negative FAB mass spectrum of **1** suggested the presence of HSO_4^-. This was confirmed by accurate mass measurement of the *m/z* 97 ion (found 96.9597, calcd 96.9596).

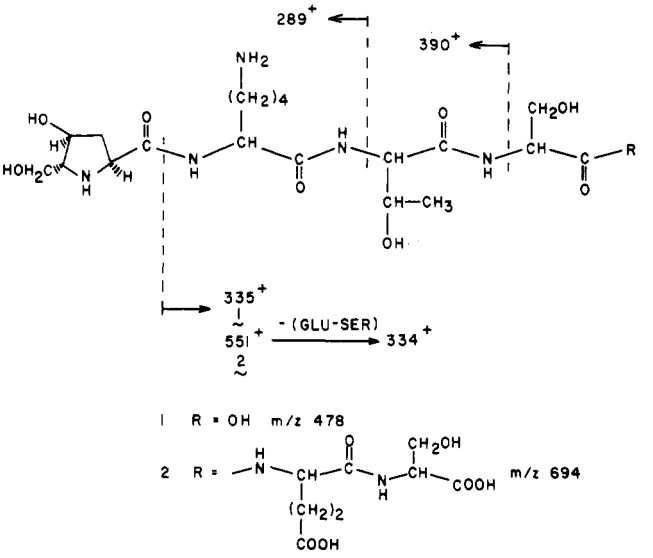
The structures of both fragments produced by unimolecular desulfonation were examined by MS/MS methods. The predominant fragmentation pathway involves the loss of both *N*-acetylglucosamine sugars. For **1**, this yields the fragments at *m/z* 681 and 478 while for **2** ions at *m/z* 897 and 694 were produced. Under high-resolution conditions the fragment at *m/z* 694 was established as 694.3235, consistent with the proposed formula for this daughter ion ($C_{27}H_{48}N_7O_{14}$, calcd 694.3259). All four fragments were also seen in the positive FAB mass spectrum. Scheme I illustrates these relations.

Both the 478^+ and 694^+ fragments maintain the peptide sequence stripped of sugar and sulfate moieties. Scheme II shows the fragmentation for both the 478^+ and 694^+ ions as evident from their MS/MS spectra. Loss of 143 μm occurs at the amide linkage between 4-hydroxy-5-(hydroxymethyl)proline and lysine with charge being retained on the lysine-containing peptide fragment. Accurate mass measurement of this 551^+ fragment yield 551.2667, which confirmed the loss of 4-hydroxy-5-(hydroxymethyl)proline (calcd 551.2676 for $C_{21}H_{39}N_6O_{11}$). All subsequent fragments seen in the MS/MS spectra of the 478^+ and 694^+ ion were indistinguishable from background in their respective mass spectra.

Scheme I



Scheme II



Knowledge of the component amino acids, their respective amounts, and the presence of similar fragments seen in the MS/MS spectra of the 478^+ and 694^+ ions (Figure 3) yielded the sequence given in Scheme II. The fragment at *m/z* 477 from the 694^+ ion of **2** is assigned as loss of Glu–Ser and is the amide analogue to the 478^+ fragment of **1**. Fragmentation at the Thr–Ser linkage as shown generates the 390^+ fragment for **2**. A fragment common to both **1** and **2** is the 289^+ daughter which corresponds to the amide of the bulgecinine–lysine dipeptide.

Treatment of **2** with a mixture of carboxypeptidase A, B, and Y in 0.1 M ammonium acetate yielded both Ser and Glu as evident from their thermospray LC/MS and MS/MS spectra. Parent ions of both Ser and Glu ($106^+/104^-$ and $148^+/146^-$, respectively) were observed at

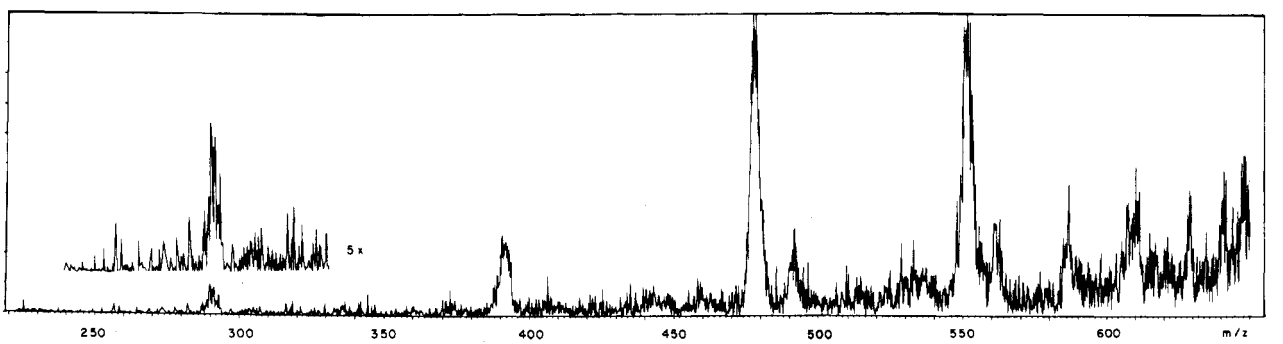


Figure 3. FAB MS/MS spectrum of the 694^+ fragment of SQ 28546.

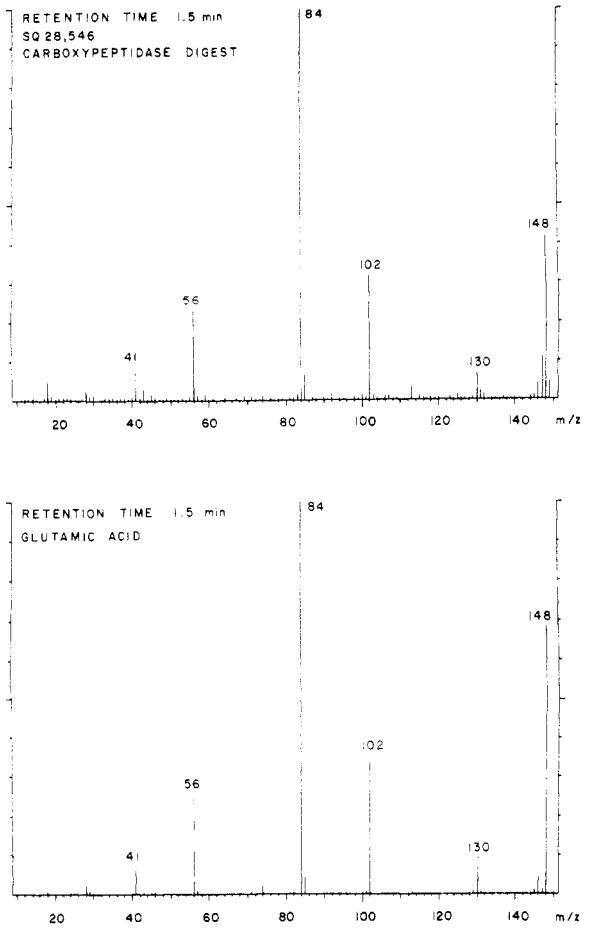
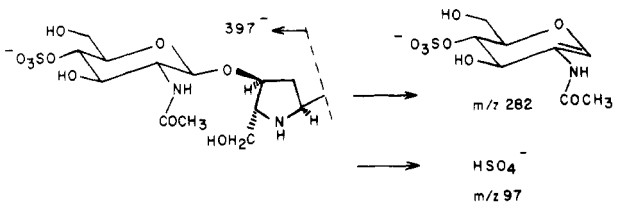


Figure 4. Thermospray LC/MS/MS spectra of (a) the carboxypeptidase digest of SQ 28546 and (b) glutamic acid.

ca. t_R 1.8 and 1.5 min. No other amino acids were seen by LC/MS. MS/MS spectra of the 106^+ and 148^+ ions matched those of the protonated molecule of authentic serine and glutamic acid (see Figure 4 for Glu). The absence of Thr or other amino acids released from the carboxy terminus supports the attachment of *N*-acetylglucosamine to threonine.

In a similar manner, mass spectral evidence established the attachment of sulfate to *N*-acetylglucosamine and showed its relation to 4-hydroxy-5-(hydroxymethyl)proline (bulgecinine). The FAB MS/MS spectrum of the 962^- parent of **1** defined the 397^- fragment. This ion was also evident from the FAB mass spectra of both **1** and **2**. Examination of this daughter established the fragmentation illustrated in Scheme III. Ions at m/z 282 and 97 were observed. As previously mentioned, the ion at m/z 97 was determined as HSO_4^- by high-resolution analysis while the m/z 282 fragment is due to sulfated *N*-acetylglucosamine. Comparison of the FAB MS/MS spectra of the 282^- and 397^- ions of **1** and **2** with those fragments from the related compound bulgecin (**4**) suggest a similar, if not identical, structure for these fragments. The negative FAB mass spectrum of bulgecin exhibited the $(M - H)^-$ ion at m/z 550. The MS/MS spectrum of this ion produced 397^- and 282^- fragments. Subsequent analysis of the 282^- fragment using FAB MS/MS indicated the 97^- daughter ion arises from this ionic species.

Scheme III



In conclusion, chemical and spectroscopic characterization of glycopeptides **1** and **2** show similarities to bulgecins; however, **1** and **2** are significantly larger peptides and contain two glucosamine residues. Both glycopeptides exhibit similar NMR and mass spectral features which have been examined in detail to define the structures shown. Mass spectral relations are best determined by using a combination of MS/MS with high-resolution analysis. The presence of structural features common to bulgecins and **1** and **2** are of biological interest. All of these compounds have been reported to potentiate the activity of β-lactam antibiotics. Furthermore, their coproduction with monobactams in broth cultures may be of interest from a biosynthetic standpoint.

Acknowledgment. We thank Dr. M. A. Porubcan and Ms. A. Kahle for NMR work. Drs. S. Shinagawa and M. Nishikawa (Takeda Chemical Industries) supplied a sample of bulgecin A for comparison studies. Helpful discussions with Dr. W. L. Parker are gratefully acknowledged.

Registry No. SQ 28504, 96827-99-5; SQ 28546, 96828-00-1.

Di- and Trisubstituted γ-Lactones. Conformational Study by Molecular Mechanics Calculations and Coupling Constant Analysis

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Received February 3, 1986

A conformational study on di- and trisubstituted γ-butyrolactones has been performed using molecular mechanics (MM) calculations and analysis of coupling constants through an empirically generalized Karplus equation. Our results corroborate the existence of only two envelope conformations for every compound. Calculated coupling constants reproduce experimental values with a global root-mean-square (rms) deviation of 0.93 Hz.

The γ-lactone system possesses great importance in natural product chemistry. The existence of several metabolites having as constitutional unit the γ-butyrolactone ring **1**, such as eldanolide, **2**, litsenolides **3**, and antimycinones **4**, has generated an enormous interest on the synthesis and configurational assignments of variously

0022-3263/86/1951-3946$01.50/0