A novel monocyclic β-lactam antibiotic SQ 26,180 has been isolated from bacteria and the structure, (R)-3-acetylamino-3-methoxy-2-oxo-1-azetidinesulfonic acid was deduced from its spectroscopic properties. Structural confirmation and assignment of absolute configuration were made by synthesis from 6-aminopenicillanic acid.

Beta-lactam antibiotics comprise one of the major chemotherapeutic means for the control of bacterial infections. This group of antibiotics has been the subject of intensive research in recent years, leading to the discovery of several new classes of naturally occurring β-lactams, namely the cephamycins, nocardicins, clavulanic acids and a burgeoning family of carbapenems. We now wish to report the isolation and characterization of a strongly acidic monocyclic β-lactam antibiotic, SQ 26,180, a member of a new class of β-lactam antibiotics, discovered independently by workers at both Takeda2 and Squibb2 for which we have suggested the generic term “monobactam”. SQ 26,180, produced by fermentations of Chromobacterium violaceum SC 11,378, was shown to have structure 1, (R)-3-acetylamino-3-methoxy-2-oxo-1-azetidinesulfonic acid. Isolation of SQ 26,180 was monitored using sensitive strains of Bacillus licheniformis and Pseudomonas aeruginosa. The characterization of the producing organism and the fermentation are described in the accompanying paper.3

SQ 26,180 was isolated from fermentation broths as outlined in Fig. 1.

The antibiotic was extracted into dichloromethane containing a tetraalkylammonium salt, cetlydimethylbenzylammonium chloride being effective for this purpose. Back extraction of SQ 26,180

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Table 1. Electrophoresis of SQ 26,180.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>Mobilitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium 0.05 M phosphate</td>
<td>7.0</td>
<td>0.88</td>
</tr>
<tr>
<td>Sodium 0.05 M phosphate</td>
<td>5.5</td>
<td>0.86</td>
</tr>
<tr>
<td>HOAc - H₂O, 1:9</td>
<td>2.2</td>
<td>0.88</td>
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<tr>
<td>HCO₂H - HOAc - H₂O, 1:3:36</td>
<td>1.8</td>
<td>0.91</td>
</tr>
</tbody>
</table>

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On Whatman No. 2 paper, 12 V/cm, 1 hour; mobilities relative to vitamin B₁₂ (0) and p-nitrobenzenesulfonate anion (1.00).
into water was accomplished by transforming the ion-pair into the sodium salt with either sodium iodide
or thiocyanate. The resulting aqueous extract was desalted by partition chromatography on Sephadex
G-10. Further purification was accomplished by ion-exchange chromatography on either DEAE cell-
lulose or Bio-Rad AG MP-1 resin followed by reverse-phase chromatography on Diaion HP20AG.
Conversion of the antibiotic to the potassium salt on Dowex 50W-X2 (K\(^+\)) gave a solid that was recrystal-
lized from aqueous methanol, giving analytically pure SQ 26,180.

Electrophoresis of SQ 26,180 (Table 1) indicates the presence of a strongly acidic function.

Elemental analysis of the potassium salt gives an empirical formula, C\(_6\)H\(_9\)N\(_2\)O\(_6\)SK. The infrared
spectrum (KBr) has a strong absorption at 1766 cm\(^{-1}\), indicative of a \(\beta\)-lactam ring. Intense amide I
(1673 cm\(^{-1}\)) and amide II (1522 cm\(^{-1}\)) peaks are evident as are characteristic peaks at 1265, 1051, and
636 cm\(^{-1}\), indicating the presence of an \(-\text{SO}_3\)\(^-\) group, the latter being consistent with the strongly acidic
nature of SQ 26,180. The location of the \(-\text{SO}_3\)\(^-\) group was further elucidated by treatment of a solu-
tion of SQ 26,180 in 2 \(\text{N HCl}\) with BaCl\(_2\) and NaNO\(_2\). Precipitation of BaSO\(_4\) indicated that the anti-
biotic contains a sulfamic acid function (the acid-hydrolysis product of the lactam presumably being the
reactive species in this case).\(^4\) The 1\(^3\)C and 1\(^{1}\)H NMR spectra (Table 2) led to the assignment of 1 (ex-
clusive of stereochemistry) as the structure of SQ 26,180.

The acetylamino group is apparent from the peaks at around 2 ppm in the 1\(^{1}\)H NMR spectrum and
and at 22.5 ppm in the 1\(^{13}\)C NMR spectrum. Similarly, a methoxyl group is indicated by peaks at around 3.4
and at 51.8 ppm in the 1\(^{1}\)H and 1\(^{13}\)C NMR spectra, respectively. The peak at 9.15 ppm (1\(^{1}\)H spectrum in
DMSO-\(d_6\)) coincides with the chemical shift of the amide proton in several cephamycin derivatives,\(^5\)
supporting the location of the acetyl and sulfo groups as shown in 1 instead of the transposed arrange-
ment. The coupling constant of 6.5 Hz (presumed negative) observed between the geminal protons at
C-4 in CD\(_3\)OD and DMSO-\(d_6\) is in accord with the coupling of \(-5.5\) Hz reported for the corresponding
position in other monocyclic \(\beta\)-lactams.\(^6\)

SQ 26,180 is converted by methanol in the presence of triethylamine to the methyl ester 2. The
chemical shift of the NHSO\(_3\)\(^-\) proton in 2, 4.35 ppm, is similar to that found for the analogous proton
in sodium cyclamate, 4.00 ppm, in the same solvent (DMSO-\(d_6\)) (unpublished observation).

Treatment of 1 with 6 \(\text{N HCl}\) at 114\(^\circ\)C for 15 hours yields an amphoteric compound that is negative
to ninhydrin but that gives a yellow color with Paul's reagent. The 1\(^{1}\)H NMR spectrum of the hydro-
chloride in D\(_2\)O [\(\delta 2.66\) (s, 3) and 7.86 ppm (s, 1)] and the UV spectra in water (pH 2.85, \(\lambda_{\text{max}}\) 215 nm,
\(\varepsilon 8900\)) and in dilute NaOH (pH 12, \(\lambda_{\text{max}}\) 238 nm, \(\varepsilon 7300\)) suggest structure 3 and this was verified by com-
parison with authentic material.\(^7,\(^8\))

<table>
<thead>
<tr>
<th>C/H</th>
<th>Solvent</th>
<th>Assignment*</th>
</tr>
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<tbody>
<tr>
<td>H (b)</td>
<td>CD(_3)OD</td>
<td>3.80, 3.92 ((J) = 6.5 Hz)</td>
</tr>
<tr>
<td>H (b)</td>
<td>DMSO-(d_6)</td>
<td>3.55, 3.69 ((J) = 6.6 Hz)</td>
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<tr>
<td>H (c)</td>
<td>D(_2)O</td>
<td>3.98</td>
</tr>
<tr>
<td>C (b, d)</td>
<td>DMSO-(d_6)</td>
<td>160.5</td>
</tr>
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</table>

(a) Chemical shifts are in parts per million downfield from (b) tetramethylsilane or (c) sodium 3-tri-
methylsilylpropionate-\(d_4\). (d) Multiplicities from a single-frequency off-resonance decoupled spectrum
are in accord with structure 1.
Confirmation of the structural assignment and determination of the C-3 stereochemistry of SQ 26,180 was accomplished by synthesis from a β-lactam antibiotic of known absolute configuration, as shown in Fig. 2. 7-ADCA, 4, was a convenient source of chiral starting material since it is prepared from 6-APA in bulk via the MORIN rearrangement. The stability of the cephem nucleus allowed use of an acid-removable protecting group in the early stages of the synthesis as part of an efficient process for the preparation of N-acetyl-7α-methoxy-7-ADCA, utilizing methodology previously reported by workers at Squibb.9) Using procedures similar to those applied to the degradation of penicillanic acids, the methoxylated cephalosporin was degraded to a monocyclic azetidinone, which upon sulfonation yielded SQ 26,180.

Sulfenylation of silylated 7-ADCA gave thiooxime 5,9) which was readily converted to benzhydryl
ester 6. Sclvolytic sulfenyl transfer rearrangement and acetylation in situ of the intermediate methoxyamine gave 7 which yielded methoxylated cephalosporin 8 after acidic cleavage of the protecting ester. Desulfurization and reduction of 8 with RANEN nickel formed desthiocepham 9a as a mixture of diastereomers. Oxidative decarboxylation was accomplished with a lead tetraacetate-cupric acetate mixture yielding isomeric acetates 9b, that were hydrolytically degraded to (R)-3-acetylamino-3-methoxy-2-azetidinone (10). Although 10 proved unstable to a variety of sulfonation conditions, a modest yield (23 %) of SQ 26,180 was obtained when 10 was treated at 0°C with the highly electrophilic sulfonating agent, DMF•SO₃ complex. The product, isolated as the Bu₄N⁺ salt 1b, was identical to naturally occurring SQ 26,180, thus confirming the structure and assigning the R-configuration at the C-3 position.

We have also isolated a related antibiotic, SQ 26,445, having a dipeptide group at the 3-position. The 3-methoxy-2-oxo-1-azetidinesulfonic acid moiety was easily recognized from the NMR and IR spectra. Acid hydrolysis gave D-glutamic acid, D-alanine and ammonia in a ratio of 2:1:2. The Glu residue was shown to be N-terminal by the SANGER method. Comparison of the ¹H NMR spectrum with data published for -Glu-Ala showed that the dipeptide side chain is linked in this manner. The structure of SQ 26,445 is thus 11.

The peculiar ratio of amino acids is presumably due to partial diversion of the hydrolysis to an imidazole analogous to 3. However, this was not isolated and characterized. Following the completion of our studies, workers at Takeda reported the isolation and structure determination of this antibiotic and named it sulfazecin.

Furthermore, a group of five monobactams having N-acetylphenylalanyl and related side chains has been isolated by us from cultures of Agrobacterium radiobacter; a more complete account of this work is forthcoming.

The diversity of naturally occurring monobactams suggests that they truly constitute a new class of antibiotics that derives activation of the β-lactam ring from the electronegative sulfonic acid residue. All members of this class isolated to date have been produced by bacterial fermentation. An investigation of synthetic monobactams has led to a highly active analog, SQ 26,776, possessing excellent β-lactamase stability and selective activity against Gram-negative organisms, including Pseudomonas aeruginosa. This analog is currently being developed for clinical evaluation.

Experimental

NMR spectra were determined on Varian Associates model XL-100-15 and T-60 and on Jeol Ltd. model FX60Q spectrometers; chemical shifts are given in ppm (δ) downfield from internal Me₄Si or Me₂SiCD₂CD₂CO₂Na. Infrared spectra were recorded on Perkin-Elmer model 257 and 621 spectrometers. Rotations were measured on a Perkin-Elmer model 141 polarimeter. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected.

Isolation of SQ 26,180 (1)

A culture broth (250 liters) of Chromobacterium violaceum was adjusted to pH 5 (H₂SO₄) and filtered using Celite. The filtrate was extracted with two 30-liter portions of 0.005 M cetyldimethylbenzylammonium chloride in CH₂Cl₂ and the combined organic phase extracted with 6 liters of 0.05 M NaI (adjusted to pH 5 with HOAc). The aqueous phase was concentrated in vacuo to 400 ml, washed with
butanol, and further concentrated to a residue. This was dissolved as much as possible in methanol and the resulting solution taken to dryness, giving 38.6 g of residue. This was chromatographed on a 2.5 × 104 cm column of Sephadex G-10 in methanol - water, 1:1, giving 5 g of residue from the active fractions. Chromatography on a 5 × 42 cm column of Whatman DE32 cellulose, eluting with a linear gradient prepared from 4 liters of pH 5 sodium 0.01 M phosphate and 4 liters of pH 5 sodium 0.1 M phosphate buffers, gave 0.46 g of active material after removal of inorganic salts by trituration with methanol and chromatography on a 2.5 × 98 cm column of Sephadex LH-20 in water. Further chromatography on a 2.5 × 66 cm column of Diaion HP20AG eluting with water gave 0.24 g of material that was chromatographically homogeneous (TLC on Merck silica gel 60, 2-BuOH - HOAc - H2O, 3:1:1, RYDON-SMITH, Rf 0.40). Conversion to the potassium salt on a 1.5 × 9 cm column of Dowex 50W-X2 (K+) gave 0.19 g of a crystalline solid that was recrystallized three times from H2O - MeOH, 1:9, to give 96 mg of analytically pure SQ 26,180, 1a, mp 194°C (dec.), [α]21D +94° (c 1, H2O).


Found: C 26.01, H 3.27, N 10.18, S 11.36, K 14.01.

The tetrabutylammonium salt was prepared by extraction of an aqueous solution of the potassium salt and one equivalent of Bu4NHSO4 with several portions of CH2Cl2. Removal of the solvent in vacuo gave 1b, [α]D = 65° (c 1, CHCl3).

Methanolysis of SQ 26,180

A solution of 49 mg of SQ 26,180 in 4 ml of MeOH and 0.04 ml of Et3N was left at room temperature for 20 hours. The solvents were removed in vacuo and the residue lyophilized, giving 50 mg of 2 as a highly deliquescent solid. Upon exposure to air, the lyophilate formed a crystalline monohydrate, mp 156~158°C (dec.): 1H NMR (DMSO-d6) δ 1.84 (s, 3), ca. 3.1 (m, CH2), 3.21 (s, 3), 3.58 (s, 3, CO2CH3), 4.32 (t, 1, J=6.7 Hz, NHSO3-) and 9.02 (s, 1, NHAc); IR (KBr) 1732 (ester C=O), 1685 (amide I), 1533 (amide II), 1209 and 1047 cm⁻¹ (-SO3-).

Sulfenimine 5

The thiooxime was prepared according to the procedure described by GORDON, et al. where 5 is isolated as the sodium salt during work-up. The sodium salt of 5 prepared from 26.34 g (123 mmole) of 7-ADCA was suspended in a water-EtOAc mixture and the pH was lowered to 2.3 with 1 N HCl. After extracting several times with EtOAc, the combined extract was dried (Na2SO4), filtered, and solvent was removed in vacuo. The residue was triturated with ether, yielding 5 as a bright yellow powder (19.62 g) that was used without further purification. Two more crops (3.23 g) were obtained for a total yield of 56%; mp 171~172°C (dec.): 1H NMR (CD3COCD3) δ 2.15 (s, 3, CH3), 2.35 (s, 3, CH3), 3.25 and 3.70 (AB quartet, J=18 Hz, 2, CH2), 5.50 (s, 1, H-6), 7.27 and 7.50 (two d's, Jortho=8 Hz, aromatic); IR (KBr) 1770 ((β-lactam C=O) and 1695 cm⁻¹ (COOH).


Found: C 53.92, H 4.19, N 8.37, S 19.32.

Sulfenimine Benzhydryl Ester 6

To a solution of acid 5 (19.0 g, 56.9 mmole) in CH2Cl2 (250 ml) was added dropwise diphenyldiazomethane (12.0 g, 61.9 mmole) in CH2Cl2. After several hours at room temperature the reaction mixture was washed twice with saturated NaHCO3 solution, dried (Na2SO4), and solvent was removed in vacuo. The residue was triturated with ether, yielding 6 as a bright yellow powder (19.62 g) that was used without further purification. Two more crops (3.23 g) were obtained for a total yield of 56%; mp 171~172°C (dec.): 1H NMR (CD3COCD3) δ 2.15 (s, 3, CH3), 2.35 (s, 3, CH3), 3.25 and 3.70 (AB quartet, J=18 Hz, 2, CH2), 5.50 (s, 1, H-6), 7.27 and 7.50 (two d's, Jortho=8 Hz, aromatic); IR (KBr) 1770 ((β-lactam C=O) and 1695 cm⁻¹ (COOH).


Found: C 66.92, H 4.86, N 5.59, S 12.88.

7-α-Acetylaminol-7-methoxydesacetoxycephalosporanic Acid Benzhydryl Ester (7)

The procedure described by GORDON et al. for direct conversion of a sulfenimine to a 7α-methoxy amide5 was used to prepare 7 from sulfenimine 6 (4 g, 8 mmole). The crude product was chromatographed on a silica gel column (250 g, Baker 60~200 mesh) eluting with EtOAc - CH2Cl2 (1:9). Impure leading and tailing fractions were combined and purified by preparative thin-layer chromatography...
The combined purified product was solidified by precipitation from an ether-pentane mixture (total yield: 1.82 g, 50%). \( ^1H \) NMR (CDCl\(_3\)) \( \delta \) 2.07 and 2.15 (two s's, 6, CH\(_3\)'s), 3.17 (broad s, 2, CH\(_2\)), 3.55 (s, 3, OCH\(_3\)), 5.07 (s, 1, H-6), 6.90 (broad s, 2, CH and NH) and 7.32 (m, 10, aromatic); IR (null) 1770 (\( \beta \)-lactam C=O), 1740 (ester C=O) and 1670 cm\(^{-1}\) (amide C=O).

7\(\beta\)-Acetylamino-7-methoxydesacetoxycephalosporanic Acid (8)

A solution of cephem ester 7 (1.67 g, 3.69 mmole) in a mixture of anisole (2 ml) and CH\(_2\)Cl\(_2\) (20 ml), cooled in an ice bath, was treated with trifluoroacetic acid (4 ml) for 4.5 hours. Solvent was removed in vacuo giving a residual oil which, after washing with pentane, was solidified and triturated with an ether-pentane mixture yielding acid 8 as a colorless powder (1.03 g, 97%): mp 183~184°C (dec.); \( ^1H \) NMR (CD\(_3\)OD) \( \delta \) 2.06 (s, 3, CH\(_3\)), 2.16 (s, 3, CH\(_3\)), 3.52 (s, 3, OCH\(_3\)), and 5.01 (s, 1, H-6); IR (KBr) 1760 (N-lactam C=O), 1740 and 1720 (COOH), 1675 (amide C=O), and 1520 cm\(^{-1}\) (amide II).


Found: C 46.19, H 5.03, N 9.38, S 11.11.

Desthiocepham 9a

A solution of methoxycephem 8 (650 mg, 2.27 mmole) and NaHCO\(_3\) (191 mg, 2.27 mmole) in water was added to a suspension of commercial RANEY nickel (11 ml of slurry, Grace No. 28 washed to neutrality with H\(_2\)O) in water (20 ml). After lowering the mixture into an oil bath pre-heated to 170°C, refluxing commenced in 2 minutes and was continued for 15 minutes. The reaction vessel was cooled rapidly in an ice bath, the catalyst was removed by filtration through Celite, and the pH was lowered to 2 with 1 N HCl. After extraction of the acidic solution with four portions of EtOAc, the combined extract was dried (Na\(_2\)SO\(_4\)). Solvent was removed in vacuo and the residue was chromatographed on silica gel (Mallinckrodt SilicAR CC-4, 1 \( \times \) 13 cm column). Elution with MeOH - CHCl\(_3\) (contains 0.75% EtOH) (1: 49) yielded 9a as a foam (381 mg, 65%); \( ^1H \) NMR (CD\(_3\)COCD\(_3\)) \( \delta \) 2.05 (s, 3, CH\(_3\)), 2.16 (s, 3, CH\(_3\)), 3.40 and 3.43 (two s's, diastereomeric OCH\(_3\)'s), and 3.68~4.20 (complex m, CH and CH\(_2\)).

Formation of Acetate 9b via Kochi Reaction

To a solution of desthiocepham 9a (514 mg, 1.99 mmole) in dry CH\(_3\)CN (15 ml), purged with argon for 15 minutes, was added cupric acetate (397 mg, 1.99 mmole), followed after 1 minute of stirring by lead tetraacetate (882 mg, 1.99 mmole). The mixture was warmed in a pre-heated oil bath at 65~75°C while continuing to purge with a stream of argon. After 15 minutes the reaction was cooled to room temperature, filtered through Celite, and solvent was removed from the filtrate under reduced pressure. The residue was combined with water and extracted with four portions of EtOAc. Drying (Na\(_2\)SO\(_4\)) of the combined organic extract and removal of solvent in vacuo gave 9b as an oil (394 mg, 73%): \( ^1H \) NMR (CDCl\(_3\)) \( \delta \) 0.97 (broad d, 6, CH\(_3\)'s), 2.08 (s, 6, CH\(_3\)CO's), 3.43 (s, 3, OCH\(_3\)), 3.58~3.65 (complex m, 2, CH\(_2\)), 5.40 (d, \( J=8 \) Hz, 1, -CHO-), and 8.03 (broad s, 1, NH); IR (CHCl\(_3\)) 1775 (\( \beta \)-lactam C=O), 1740 and 1720 (COOH), 1675 (amide C=O), and 1520 cm\(^{-1}\) (amide II).

(R)-3-Acetylamino-3-methoxy-2-azetidinone (10)

Acetate 9b (394 mg, 1.45 mmole), dissolved in a MeOH (10 ml) and water (1 ml) mixture and cooled in an ice-MeOH bath at ~15 to ~10°C, was treated with solid K\(_2\)CO\(_3\) (200 mg, 1.45 mmole) and sodium borohydride (55 mg, 1.45 mmole). After stirring in the cold bath for 2 hours solvent was removed in vacuo. The residue was dissolved in water, the pH was lowered to 6 with 1 N HCl and water was removed under reduced pressure. Extraction of the solid residue with acetone gave the product as an oil, which was purified on silica gel (4 g of Mallinckrodt SilicAR CC-4), eluting with MeOH - CH\(_3\)Cl\(_2\) (1: 19). Crystallization of the product with ether gave 10 as a colorless powder (141 mg, 62%), mp 103~113.5°C. Recrystallization from acetone - ether gave analytically pure material, mp 112~113°C: \( ^1H \) NMR (CDCl\(_3\)) \( \delta \) 2.08 (s, 3, CH\(_3\)CO), 3.50 (s, 3, CH\(_3\)O), 3.72, 3.90 (AB quartet, 2, \( J=6.5 \) Hz, CH\(_2\)), 5.98 (broad, 1, NH) and 6.79 (broad, 1, NH); IR (KBr) 1760 (\( \beta \)-lactam C=O), 1665 (amide C=O), and 1523 cm\(^{-1}\) (amide II).

Anal. Calcd. for C\(_6\)H\(_{10}\)N\(_2\)O\(_3\): C 45.56, H 6.37, N 17.72.

Found: C 45.48, H 6.45, N 17.68.
Synthetic SQ 26,180 as the Bu₄N⁺ Salt (1b)

DMF•SO₃ complex, freshly prepared from trimethylsilyl chlorosulfonate and dry DMF (cf. Ref. 15), was used as a 1 M solution in DMF. (R)-3-Acetylamino-3-methoxy-2-azetidinone (10) (40 mg, 0.254 mmole), placed in a flask cooled in an ice-bath, was treated with the above stock solution (1 ml) and, after 5 minutes, was poured into 0.5 M KH₂PO₄. After washing the aqueous solution with CH₂Cl₂, tetrabutylammonium bisulfate (121 mg, 0.25 mmole) was added and the ion-paired product was extracted with CH₂Cl₂ giving an oil. Chromatography on silica gel (3.5 g, Mallinckrodt SilicAR CC-4), eluting with MeOH - CH₂Cl₂ (3: 97), yielded 1b as a foam (28 mg, 23%), [α]D +61.5° (c 0.33, CHCl₃). The product was identical to the Bu₄N⁺ salt of SQ 26,180 from fermentation broths by TLC [silica gel, E. Merck 60F, EtOAc - MeOH (2: 1)], ¹H NMR (CDCl₃, 100 MHz), and ¹³C NMR (CDCl₃).

References