Tetrahedron Vol 40, No 18, pp 3465 to 3470, 1984
Printed in the U S A

0040-4020/84 $3.00 + .00
Pergamon Press Ltd

STRUCTURES OF BULGECINS, BACTERIAL METABOLITES WITH BULGE-INDUCING ACTIVITY

SUSUMU SHINAGAWA,* FUMIKO KASAHARA, YOSHIKAZU WADA, SETSUO HARADA and MITSUKO ASAI
Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka 532, Japan

(*Received in Japan* 4 *November* 1983)

Abstract—The structure of bulgecin A, a bacterial metabolite with bulge-inducing activity, was determined chemically and spectrometrically to be 4-O-(2′-acetylamino-2′-deoxy-4′-O-hydroxysulfonyl-β-D-glucopyranosyl)-(2*S*, 4*S*, 5*R*)-4-hydroxy-5-hydroxymethylprolyltaurine monosodium salt. Bulgecins B and C, the minor components, were found to be analogs of bulgecin A in which the taurine moiety was replaced as shown in Fig. 1.

Pseudomonas acidophila strain G-6302 and *Pseudomonas mesoacidophila* strain SB-72310 produce monocyclic β-lactams, sulfazecin[1,2] and isosulfazecin,[1,3] respectively, as shown in Fig. 2. The same organisms were found to produce new glycopeptides, bulgecins, which induce bulge-formation in cooperation with β-lactam antibiotics and enhance the lytic activity of β-lactam antibiotics.[4] Bulgecins A(1), B(2) and C(3) have been isolated and chemically characterized.[5] The present paper deals with the structural elucidation of bulgecins (Fig. 1).

Bulgecins are water-soluble acidic compounds and were isolated as monosodium salts; 1, $C_{16}H_{28}N_3O_{14}S_2Na \cdot H_2O$, colorless needles, m.p. 211–212° (dec.), $[\alpha]_D^{20} + 6.5°$ (N AcOH); 2, $C_{17}H_{28}N_3O_{13}SNa \cdot H_2O$, a white powder, $[\alpha]_D^{20} - 2.6°$ (N AcOH), 3, $C_{14}H_{23}N_2O_{12}SNa \cdot 1/2H_2O$, a white powder, $[\alpha]_D^{20} + 2.9°$ (N AcOH). The molecular formulae of 1, 2 and 3 were determined on the basis of microanalysis and mass spectrometry. The three components showed end absorption in the UV spectra. The IR spectra indicated the presence of an amide group (around 1660 and 1560 cm^{-1}) and a sulfate (around 1240 and 820 cm^{-1}). The ^{1}H NMR spectrum of 1 revealed signals of N-acetyl Me protons (δ 1.84 ppm), an anomeric proton (δ 4.45 ppm, J = 8.0 Hz) and two amide protons (δ 7.76, 8.30 ppm). Other components exhibited ^{1}H NMR spectra similar to that of 1, except that only one amide proton was observed in the case of 3. The ^{13}C NMR spectra of 1, 2 and 3 showed 16, 17 and 14 C signals, respectively, and all of the compounds exhibited an anomeric C signal at δ 101 ppm (*d*).

On acid hydrolysis with 6N HCl, each of the three components yielded D-glucosamine and a new amino acid (4) in an equimolar ratio. In addition, taurine and β-alanine were recovered in the case of 1 and 2, respectively.[5]

In order to elucidate structures of bulgecins, we first carried out structural studies on the novel amino acid (4), a common constituent of bulgecins. The compound 4, $C_6H_{11}NO_4$ (FAB-MS, $(M + H)^+$ *m/e* 162), colorless prisms, m.p. 182°, $[\alpha]_D^{24} - 13.1°$ (H_2O), pKa′ 2.6 ($-COO^-$), 8.5 ($>NH_2^+$ or $-NH_2^+-$) showed a pink-yellow color reaction to ninhydrin, similar to that of a proline derivative.[5]

The ^{13}C NMR spectrum of 4 in D_2O at 25M Hz confirmed the presence of six C atoms and the signals at 174.4 (s), 71.3 (d), 58.8 (t), 37.3 (t) ppm were readily assigned to –COOH, >CH–OH, CH_2OH and $>C–CH_2–C<$, respectively. Two residual signals at 67.6 (d) and 60.1 (d) ppm seemed to attribute to the resonances of N-methine carbons, comparing with the ^{13}C NMR spectra of proline,[6] 4-hydroxyproline,[6] and 3-hydroxy-4-methylproline.[7] These results suggested a 5-membered N-containing ring having a carboxyl group at C-2, a hydroxymethyl group at C-5 and an OH group at C-3 or C-4.

From proton spin-decoupling studies of 4, all the protons were assigned as shown in Table 1. The proton couplings between H-2 and H-3, and between H-3 and H-4 indicated the presence of the 3-methylene group in the pyrrolidine ring and established the structure of 4 as 4-hydroxy-5-hydroxymethylproline.

The L-configuration at C-2 was suggested by the more positive optical rotation of the hydrochloride ($[\alpha]_D^{24} + 12.4°$ (*c* = 0.95 N HCl)) compared with the free amino acid ($[\alpha]_D^{24} - 13.1°$ (*c* = 0.95, H_2O)) ac-

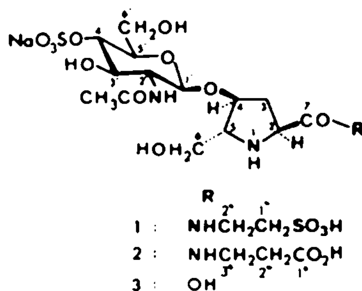

Fig. 1. Structures of bulgecins.

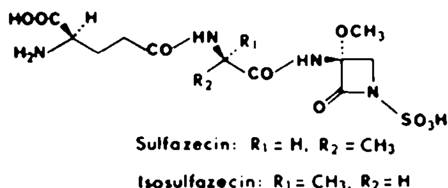

Fig. 2. Structures of sulfazecin and isosulfazecin.

Table 1. Chemical shifts and coupling constants in the ^1H NMR spectrum of **4** in D_2O at 400 MHz

Proton	δ (ppm)	J (Hz)
H-2	4.21 (dd)	$J_{2,3}$=9.0 and 6.6
H-3	2.66 (ddd)	J_{gem}=13.9, $J_{2,3}$=9.0, $J_{3,4}$=5.8
	2.16 (ddd)	J_{gem}=13.9, $J_{2,3}$=6.6, $J_{3,4}$=5.1
H-4	4.39 (ddd)	$J_{3,4}$=5.8 and 5.1, $J_{4,5}$=4.2
H-5	3.76 (m)	$J_{5,6}$=6.5 and 6.0, $J_{4,5}$=4.2
H-6	3.75 (m)	J_{gem}=14.0, $J_{5,6}$=6.0
	3.90 (dd)	J_{gem}=14.0, $J_{5,6}$=6.5

cording to the Lutz rule.[8] The positive Cotton effect ($[\Theta]_{209}$ + 426 (H_2O)) in the CD spectrum of **4** confirmed the L-configuration.[9] Among the four possible configurations for **4**, the 2,4-*cis*-2,5-*trans*-stereochemistry was established by the following chemical procedure.

N-Carbobenzoxylation of **4** with carbobenzoxy chloride (Cbz–Cl) gave N-Cbz-derivative (**5**). Acetylation of **5** with Ac_2O-pyridine gave N-Cbz, di-O-acetate (**6**), $C_{18}H_{21}NO_8$ (MS, M^+ *m/e* 379), which confirmed the presence of two OH groups in the molecule. When N-hydroxy-5-norbornene-2,3-dicarboximide (HONB) ester of **5**, which was prepared by the dicyclohexylcarbodiimide (DCC) method,[10] was allowed to stand in CH_3CN in the presence of triethylamine, the γ-lactone of **5** (**7**) was formed in a good yield. The IR spectrum of **7** showed an intense peak at 1800 cm^{-1} (γ-lactone), similar to that observed for Cbz-*allo*-hydroxy-proline lactone.[11] An apparent downfield shift at H-4 (about 0.7 ppm) by lactonization was also revealed in the ^1H-NMR spectrum of **7**. γ-Lactone formation has been observed for *cis*-4-hydroxyproline, and not for *trans*-4-hydroxyproline.[11,12] Therefore, the selective formation of γ-lactone indicated that the carboxyl group was on the same side of the ring as the 4-OH group, and on the opposite side to the 5-hydroxymethyl group as shown in Fig. 3. The (2*S*, 4*S*, 5*R*-configuration of **4** was finally established by X-ray analysis of a degraded compound of **1**, which is described later.

Mild methanolysis and alkaline hydrolysis of **1** was carried out, respectively, to obtain partially degraded compounds. Methanolysis of **1** with 1N HCl-MeOH gave methyl α- and β-glycosides of N-acetyl-D-glucosamine (**8a,b**), desulfated bulgecin A (**9**) and a ninhydrin positive compound (**10**). Compound **9**, $C_{16}H_{29}N_3O_{11}S$, FAB MS $(M+H)^+$ *m/e* 472, gave D-glucosamine, taurine and **4** in an equimolar ratio on acid hydrolysis (Table 2), and lacked the IR absorption bands at 1220 and 820 cm^{-1} due to sulfate. Compound **10**, $C_8H_{16}N_2O_6S$, FAB MS $(M+H)^+$ *m/e* 269, gave taurine and **4** in an equimolar ratio on acid hydrolysis (Table 2). The IR spectrum of **10** showed the presence of amide (1670 and 1550 cm^{-1}) and sulfonate (br 1200 cm^{-1}), indicating the structure to be 4-hydroxy-5-hydroxymethylprolyltaurine (Fig. 4).

The above results suggested a glycosidic linkage between the amino sugar and the dipeptide (**10**). Table 3 shows the ^{13}C NMR spectra of **9** and **10**. C signals of these compounds were assigned from the ^{13}C NMR spectral data of **8b**† and **4**. Comparison of the spectrum of **9** with that of **10** showed that the signal assigned to C-4 in **9** shifts downfield by 7.7 ppm relative to that in **10**. Considering that a glycosyl unit causes a downfield shift (7–10 ppm) at the C of attachment,[13] the glycosidic linkage in **1** should exist between the anomeric C (C-1′) of the

†Assignment of C signals in **8b** was confirmed with those of glucosamine hydrochloride in Ref. 6.

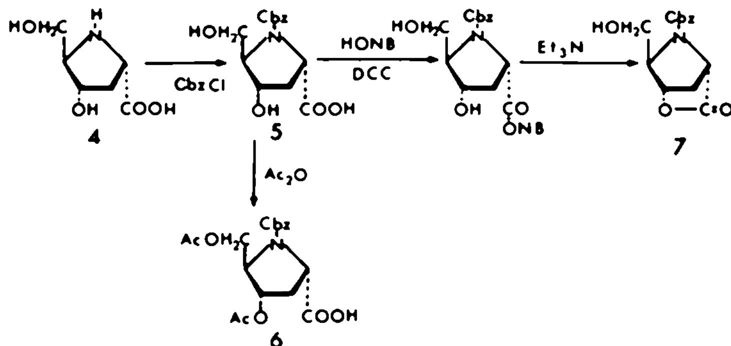

Fig. 3.

Table 2. Ratios of constitutents of bulgecins and their degraded compounds

	1	**2**	**3**	**9**	**10**	**11**	**12**
D-glucosamine	1.07	1.00	1.00	0.92	0.00	0.00	1.00
4	0.82	0.94	0.92	0.85	0.88	0.94	0.93
taurine	1.00	0.00	0.00	1.00	1.00	1.00	0.00
β-alanine	0.00	1.00	0.00	0.00	0.00	0.00	0.00
Recovery (%)	88	85	87	85	86	91	82

Compounds were hydrolyzed with 6N HCl at 110°C for 18 hr. Analysis of hydrolysates were carried out on Hitachi 835 amino acid analyzer.

amino sugar and C-4 of the amino acid moiety. The configuration of the glycosidic linkage was confirmed to be the β form from the following spectral data: the chemical shifts of the anomeric C (C-1′) in **1** (101.1 ppm) and **9** (101.3 ppm) were more consistent with that of methyl β-glycoside (**8b**, 102.7 ppm) than that of methyl α-glycoside (**8a**, 98.8 ppm); the coupling constant (J = 8.0 Hz) between H-1′ (anomeric proton) and H-2′ in the ^{1}H NMR spectrum of **1**[5] indicated that these protons are axial.

The location of sulfate was determined by comparing the ^{13}C NMR spectrum of **1** with that of desulfated bulgecin A (**9**, Fig. 4). Taking account of the report by Honda *et al.*[14] that O-sulfation of carbohydrates causes a marked downfield shift (8–10 ppm) at the α-C and moderate upfield shifts (2 3 ppm) at the β-carbons, C signals in the ^{13}C NMR spectrum of **1** were assigned as shown in Table 3, which indicated that the sulfate is located at C-4′ of the sugar moiety. Neither the 3′ O– nor the 6′ O-sulfate structure explained the ^{13}C NMR spectrum of **1**.

From all of these results, the structure of **1** was determined as that shown in Fig. 1.

Alkaline hydrolysis of **1** with 0.2N $Ba(OH)_2$ gave a desulfated derivative (**11**). Compound **11**, colorless

Table 3. ^{13}C NMR spectra of bulgecins and their degraded compounds in D_2O at 25 MHz

Carbon	**4**	**8b***	**10**	**9**	**1**	**2****	**3**	**12**
C-2	60.1(d)		59.0(d)	59.4(d)	59.5(d)	59.2(d)	60.4(d)	60.4(d)
C-3	37.3(t)		37.8(t)	36.7(t)	36.7(t)	36.7(t)	35.9(t)	35.9(t)
C-4	71.3(d)		71.3(d)	79.0(d)	79.1(d)	79.7(d)	79.7(d)	79.6(d)
C-5	67.6(d)		68.0(d)	66.2(d)	66.2(d)	65.9(d)	65.9(d)	65.9(d)
C-6	58.8(t)		58.8(t)	59.0(t)	59.0(t)	59.6(t)	58.9(t)	58.8(t)
C-7	174.4(s)		169.3(s)	168.7(s)	168.8(s)	170.5(s)	174.0(s)	174.0(s)
C-1′		102.7(d)		101.3(d)	101.1(d)	101.3(d)	101.4(d)	101.50(d)
C-2′		56.3(d)		56.2(d)	56.0(d)	56.1(d)	56.1(d)	56.4(d)
C-3′		74.9(d)		74.5(d)	73.1(d)	73.0(d)	72.9(d)	74.3(d)
C-4′		70.9(d)		70.8(d)	77.5(d)	77.7(d)	77.6(d)	70.7(d)
C-5′		76.8(d)		76.8(d)	75.2(d)	75.3(d)	75.1(d)	76.7(d)
C-6′		61.7(t)		61.6(t)	61.3(t)	61.3(t)	61.3(t)	61.6(t)
$\underline{C}OCH_3$		175.0(s)		174.8(s)	174.9(s)	175.3(s)	175.2(s)	175.1(s)
$CO\underline{C}H_3$		23.1(q)		23.2(q)	23.2(q)	23.1(q)	23.1(q)	23.1(q)
C-1″			50.4(t)	50.4(t)	50.4(t)	180.6(s)		
C-2″			36.5(t)	36.5(t)	36.5(t)	37.8(t)		
C-3″						37.2(t)		

* OCH_3: 57.8(q). ** Measured at 100 MHz.

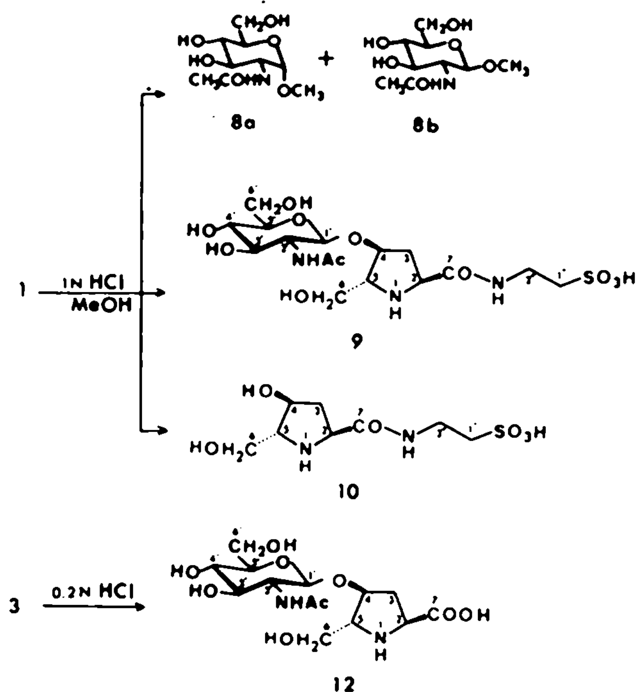

Fig. 4.

prisms, $C_{16}H_{29}N_3O_{11}S \cdot H_2O$, had a different IR spectrum and specific rotation value from those of desulfated bulgecin A (9). On acid hydrolysis, 11 gave taurine and 4, but not D-glucosamine (Table 2). The structure determination of 11 was accomplished by X-ray crystallographic analysis. Compound 11 was crystallized from aqueous MeOH in the triclinic space group P1 with a = 8.620 (3), b = 8.898 (5), c = 8.100 (3) Å, α = 111.86 (3), β = 90.27 (3), γ = 72.11 (3)° and one molecule of $C_{16}H_{29}N_3O_{11}S \cdot H_2O$ in the unit cell. The structure was solved as described in the Experimental. Fig. 5 shows a perspective view of the molecule of 11.

As shown in Fig. 5, 11 has a 2-acetylamino-2-deoxy-D-gulose moiety instead of acetylglucosamine in 9. It was assumed that double inversions of the configuration at both C-3' and C-4' of 1 occurred by alkaline solvolysis of sulfate (Fig. 6).[15]

Structures of 2 and 3 were determined by comparing the ^{13}C NMR spectra of 2 and 3 with that of 1. All C signals of 2 and 3 were assigned as shown in Table 3. Consequently, the terminal taurine moiety in 1 was replaced by β-alanine in 2 and lost in 3 (Fig. 1). Bulgecin C (3), on mild acid hydrolysis, gave desulfated bulgecin C (12), $C_{14}H_{24}N_2O_9$, colorless needles, m.p. 235–238° (dec.) (Fig. 4). Comparison of the ^{13}C NMR spectrum of 12 with that of desulfated bulgecin A (9) showed that the chemical shifts of the

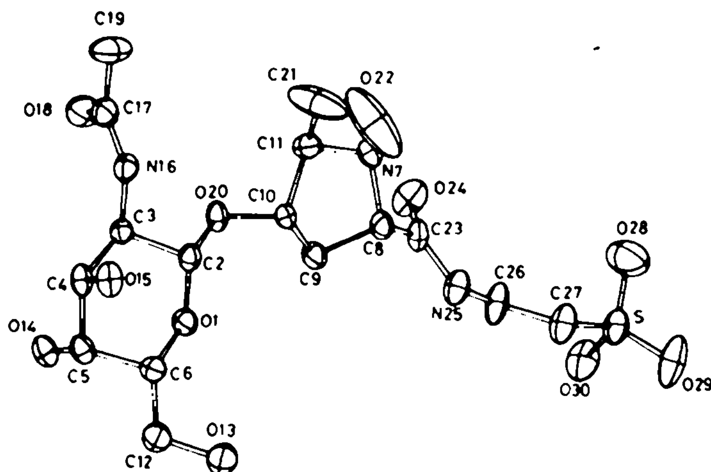

Fig. 5. Perspective view of 11.

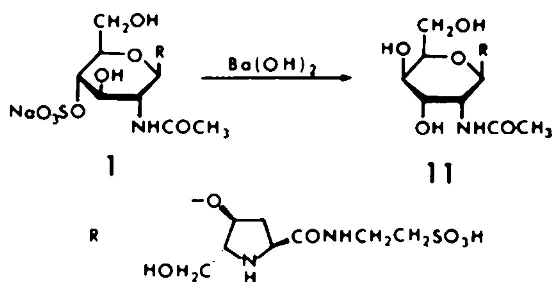

Fig. 6. Alkaline solvolysis of sulfate in 1.

carbons in 12 agreed well with those of the corresponding carbons in 9 (Table 3), supporting the structure of 3.

Bulgecins are new glycopeptides having a unique structure and interesting activity. 4-Hydroxy-5-hydroxymethylproline, a common constituent of bulgecins, is a new cyclic imino acid, although a number of proline or pipecolic acid derivatives have been identified so far in natural sources. It is noteworthy from a biosynthetical point of view that bulgecin and sulfazecin, both of which are produced by the same bacterium, have a sulfate group in the molecule, even if N-sulfate in the case of sulfazecin. Further studies on biological activities and structure-activity relations will be described in the future.

EXPERIMENTAL

M.p. determinations were performed with Yamato model MP-21 m.p. apparatus. IR spectra were recorded with Hitachi 285 grating IR spectrophotometer. Rotations were determined with Jasco DIP-181, and CD spectra were recorded with Jasco ORD/UV-5. NMR spectra were obtained using Varian XL-100, EM-390 or JNM-GX 400 instrument; chemical shift (δ) are reported in ppm down field from an internal TMS reference. Mass spectra were determined with JEOL JMS-DX 300 or JEOL JMS-01SG mass spectrometer.

N-Cbz-derivative of 4 (5). To a soln of 4 (161 mg) in water (10 ml), Cbz Cl (210 mg) and $NaHCO_3$ (240 mg) were added at 0°. After stirring at 0° for 1 hr and at room temp. overnight, the soln was washed with ether. The aqueous layer was neutralized with N HCl and to this was added AcOEt (20 ml). The AcOEt soln was washed with sat NaCl aq, dried over Na_2SO_4 and evaporated to give 250 mg of 5 as an oil. This was dissolved in AcOEt (10 ml) together with dicyclohexylamine (DCHA, 0.18 ml). The resulting crystals were collected by filtration to give 395 mg of DCHA salt of 5, m.p. 203–204°, $[\alpha]_D^{24}$ + 11.8° (c = 1.0, DMF). (Found: C, 65.63; H, 8.32; N, 6.10. Calc for $C_{14}H_{17}N_1O_6 \cdot C_{12}H_{23}N$: C, 65.52; H, 8.46; N, 5.88%.)

N-Cbz-di-O-acetate of 4 (6). The soln of DCHA salt of 5 (365 mg) in AcOEt (10 ml) was washed with 0.1N H_2SO_4 (10 ml), and dried over Na_2SO_4. To the AcOEt soln was added Ac_2O (0.2 ml) and pyridine (1 ml). The mixture was stirred at room temp. for 3 hr, and poured into 5% $NaHCO_3$ aq (20 ml). The aqueous layer was neutralized with N HCl and extracted with AcOEt (20 ml). The AcOEt layer was washed with water, dried over Na_2SO_4 and evaporated to give 410 mg of 6 as an oil (MS, M^+ m/e 379). This was dissolved in AcOEt (10 ml) together with DCHA (0.14 ml). The resulting crystals were collected to give 390 mg of DCHA salt of 6, m.p. 194–195°, $[\alpha]_D^{24}$ − 19.8° (c = 0.5, DMF). (Found: C, 64.17; H, 7.76; N, 4.89. Calc for $C_{18}H_{21}NO_8 \cdot C_{12}H_{23}N$: C, 64.26; H, 7.91; N, 5.00%.)

N-Cbz-4-hydroxy-5-hydroxymethylproline lactone (7). The soln of DCHA salt of 5 (950 mg) in AcOEt (20 ml) was washed with 0.1N H_2SO_4 (20 ml), dried over Na_2SO_4 and evaporated. The resulting oil was dissolved in CH_3CN (10 ml), and to this was added N-hydroxy-5-norbornene-2,3-dicarboximide (HONB) (395 mg) and dicyclohexylcarbodiimide (440 mg) at 0°. The mixture was stirred at 0° for 4 hr and filtered to remove insolubles. To the filtrate was added Et_3N (0.28 ml) and the mixture was kept at room temp. overnight and evaporated. The residue was extracted with AcOEt (100 ml), which was washed with water and dried over Na_2SO_4. AcOEt was evaporated to give 7 as an oil (580 mg), which gave a single spot on TLC. TLC (silica gel, Merck precoated plate), Rf = 0.31 ($CHCl_3$–MeOH = 19:1); IR, $\nu_{max}^{CHCl_3}$ 1800, 1710 cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz) δ (ppm): 3.8 (3H, m, H-5, H-6), 4.55 (1H, m, H-2), 4.97 (1H, m, H-4). (Found: C, 60.84; H, 5.72; N, 5.08. Calc for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05%.)

Methanolysis of 1. Compound 1 (5.9 g) in N HCl–MeOH (150 ml) was refluxed for 4 hr. The mixture was evaporated *in vacuo* and the residue was dissolved in water (100 ml). The soln was passed through a column (100 ml) of Amberlite IR-45 (OH^-). The effluent was successively passed through a column (100 ml) of Amberlite IRC-50 (H^+). The effluent was diluted with water to 500 ml and applied to a column (100 ml) of Amberlite IRA-68 (OH^-). The column was washed with water (100 ml) and eluted with N AcOH. The passed soln was evaporated to give a solid of a mixture of glycosides (checked by TLC), which was crystallized from MeOH–AcOEt; 820 mg. The crystals were dissolved in a mixture of $CHCl_3$–MeOH–H_2O (8:2:0.2) and the soln was applied on a silica gel column (silica gel 40 g). The column was developed with the same solvent and the fractions from 240 to 420 g were collected and evaporated. The resulting crystals were recrystallized from MeOH–AcOEt to give needles of 8a; 440 mg, m.p. 194°, $[\alpha]_D^{23}$ + 140.0° (c = 0.50, H_2O). ^{1}H NMR (100 MHz, D_2O) δ (ppm): 2.09 (3H, s, $COCH_3$), 3.44 (3H, s, OCH_3), 3.50–4.00 (6H, N,O-methine), 4.80 (1H, d, J = 3.8 Hz, anomeric proton). ^{13}C NMR (25 MHz, D_2O) δ (ppm): 22.9 (q), 54.5 (d), 55.9 (q), 61.5 (t), 70.9 (d), 72.0 (d) 72.6 (d), 98.9 (d), 174.6 (s). (Found: C, 45.79; H, 7.10; N, 5.77. Calc for $C_9H_{17}NO_6$: C, 45.95; H, 7.28; N, 5.96%.) The fractions from 480 to 680 g on the silica gel chromatography described above were collected and evaporated to give crystals, which were recrystallized from MeOH–AcOEt to give needles of 8b; 120 mg, m.p. 192–193°, $[\alpha]_D^{23}$ − 41.8° (c = 0.50, H_2O). ^{1}H NMR (100 MHz, D_2O) δ (ppm): 2.07 (3H, s, $COCH_3$), 3.53 (3H, s, OCH_3), 3.50–4.10 (6H, N,O-methine, O-methylene), 4.47 (1H, d, J = 7.8 Hz, anomeric proton.) ^{13}C NMR (25 MHz, D_2O): see Table 3. (Found: C, 46.17; H, 7.40; N, 5.89. Calc for $C_9H_{17}NO_6$: C, 45.95; H, 7.28; N, 5.96%.)

The eluate with N AcOH from the Amberlite IRA-68 column described above were concentrated. The residue was dissolved in water (10 ml) and applied on a column (3.3 × 50 cm) of Sephadex LH-20, which was developed with 10% aqueous MeOH. The fractions from 210 to 230 ml were collected, concentrated and lyophilized. The resulting powder was treated with EtOH to give crystalline solid of 9; 780 mg, $[\alpha]_D^{23}$ + 1.4° (c = 1.0, N AcOH). ^{13}C NMR (25 MHz, D_2O): see Table 3. (Found: C, 38.91; H, 6.42; N, 8.47; S, 6.16. Calc for $C_{16}H_{29}N_3O_{11}S \cdot H_2O$: C, 39.25; H, 6.38; N, 8.59; S, 6.55%.)

The fractions from 231 to 250 ml on the Sephadex LH-20 chromatography described above were collected, evaporated and lyophilized. The resulting solid was crystallized from MeOH–EtOH, and recrystallized from aqueous MeOH to give needles of 10; 520 mg, m.p. 164–166°, $[\alpha]_D^{23}$ − 10.2° (c = 0.50, N AcOH). ^{1}H NMR (100 MHz, D_2O) δ (ppm): 2.10 (1H, ddd, J = 5.0, 6.4, 14 Hz), 2.79 (1H, ddd, 6.0, 8.0, 14 Hz), 3.16 (2H, t, J = 6.4 Hz), 3.70 (2H, t, J = 6.4 Hz), 3.70–4.10 (3H, N-methine, O-methylene), 4.52 (1H, dd, J = 6.4, 8.0 Hz). ^{13}C NMR (25 MHz, D_2O): see Table 3. (Found: C, 35.98; H, 5.95; N, 10.26; S, 11.96. Calc. for $C_8H_{16}N_2O_6S$: C, 35.81; H, 6.01; N, 10.44; S, 11.95%.)

Alkaline hydrolysis of 1. To a soln of 1 (1.0 g) in water

(10 ml) was added 0.2N $Ba(OH)_2$ (50 ml), and the mixture was allowed to stand at 70° for 5 hr and at room temp. overnight. The ppts ($BaSO_4$) was collected by filtration to give 290 mg of colorless powder, and the filtrate was applied on a column (25 ml) of Amberlite IR-120 (H^+). The column was washed with water and eluted with N NH_4OH. The fractions showing positive Greig–Leaback reaction were combined and concentrated. The residue was treated with MeOH to give crystals of **11**, which were recrystallized from MeOH–H_2O to afford 340 mg of colorless prisms, m.p. 194–195°, $[\alpha]_D^{22}$ − 19.2° (*c* = 0.51, N AcOH). ^1H NMR (100 MHz, d_6-DMSO) δ (ppm): 1.85 (3H, s, $COCH_3$), 2.5–4.4 (N,O-methine, O-methylene), 4.58 (1H, d, J = 8.0 Hz, anomeric proton), 7.55 (1H, d, NHCO), 8.33 (1H, t, NHCO). (Found: C, 39.15; H, 6.43; N, 8.42; S, 6.24. Calc for $C_{16}H_{29}N_3O_{11}S{\cdot}H_2O$: C, 39.25; H, 6.38; N, 8.59; S, 6.55%.)

X-ray analysis of **11**. A Rigaku automatic four-circle diffractometer was used for data collection with graphite-monochromated MoKα radiation (λ = 0.7107 Å). Among 1869 independent reflexions measured in the range of 3° ≤ 2Θ ≤ 50°, 1777 satisfied the condition $F_0 \geq 3\sigma(F_0)$ and were used in the following calculations. Both the direct method[16] and the heavy atom method were used to determine the coordinate of 32 nonhydrogen atoms. Several steps of refinement by the least squares method[17] applying anisotropic thermal parameters to these atoms revealed 30 hydrogen atoms on the difference Fourier map. A hydrogen connected to O (22) could not be determined because of its large thermal vibration. Further refinement with isotropic thermal parameters to the hydrogen atoms converged to the final value of R = 0.039.[18]

Mild acid hydrolysis of **3**. Compound **3** (560 mg) was dissolved in 0.2N HCl (50 ml) and kept at 95° for 3 hr. After cooling, the soln was neutralized by addition of N NaOH (10 ml) and passed through a charcoal column (190 ml). The column was washed with water and eluted with 7% aqueous isobutanol. The eluate was evaporated to a small volume (15 ml) and applied on a column (100 ml) of QAE-Sephadex A-25 (Cl^-), which was developed with 0.0[illegible] M phosphate buffer (pH 6.2). The fractions from 80 to 160 ml were collected and passed through a column of Dowex 50 W × 2 (H^+, 25 ml), which was washed with water and eluted with 0.5N NH_4OH (250 ml). The eluate was concentrated and lyophilized. The resulting powder (280 mg) was treated with H_2O–MeOH to give colorless needles of **12**, m.p. 235–238° (dec.), $[\alpha]_D^{23}$ − 4.1° (*c* = 0.54, H_2O). ^{13}C-NMR (25 MHz, D_2O): see Table 3. Amino acid analysis: see Table 2. (Found: C, 46.00; H, 6.69; N, 7.62. Calc for $C_{14}H_{24}N_2O_9$: C, 46.14; H, 6.63; N, 7.68%.)

Acknowledgement—We are grateful to Drs. K. Morita, M. Yoneda and T. Kishi for their interest throughout this work. Thanks are also due to the members of Chemistry Laboratories for physicochemical analyses, to Miss K. Terao for amino acid analysis and to Messers M. Maki and Y. Nohara for their excellent technical assistance.

REFERENCES

[1] A. Imada, K. Kitano, K. Kintaka, M. Muroi and M. Asai, *Nature* **289**, 590 (1981).

[2] M. Asai, K. Haibara, M. Muroi, K. Kintaka and T. Kinshi, *J. Antibiotics* **34**, 621 (1981).

[3] K. Kintaka, K. Haibara, M. Asai and A. Imada, *Ibid.* **34**, 1081 (1981); The absolute configuration at the 3-position in isosulfazecin was determined to be *R* on the basis of the Cotton effect at $[\Theta]_{234}$ + 5970 (H_2O) in isosulfazecin and at $[\Theta]_{235}$ + 7930 (H_2O) in sulfazecin.

[4] A. Imada, K. Kintaka, M. Nakao and S. Shinagawa, *Ibid.* **35**, 1400 (1982).

[5] S. Shinagawa, M. Maki, K. Kintaka, A. Imada and M. Asai, *Ibid.*, submitted.

[6] L. F. Johnson and W. C. Jankowski, *Carbon*-13 *NMR Spectra*, pp. 117–118. Wiley, New York, (1972).

[7] F. Benz, F. Knusel, J. Nuesh, H. Treichler, W. Voser, R. Nyfeler and W. Keller-Schierlein, *Helv.* **57**, 2459 (1974).

[8] O. Lutz and B. Jirgensons, *Ber. Dtsch. Chim. bes* **63**, 448 (1930).

[9] L. Fowden, P. M. Scopes and R. N. Thomas, *J. Chem. Soc.* (C) **1971**, 833.

[10] M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa and O. Nishimura, *Chem. Pharm. Bull.* **22**, 1857 (1974).

[11] A. A. Patchett and B. Witkop, *J. Am. Chem. Soc.* **79**, 185 (1957).

[12] T. Kaneko, *J. Chem. Soc. Japan* **61**, 207 (1940).

[13] D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.* **93**, 4463 (1971).

[14] S. Honda, H. Yuki and K. Takiura, *Carbohyd. Res.* **28**, 150 (1973).

[15] M. Parquet and P. Sinay, *Ibid.* **18**, 195 (1971).

[16] Program "MULTAN" was used. G. Germain, P. Main and M. M. Woolfson, *Acta Crystallogr.* A **27**, 368 (1971).

[17] Program "X-ray System" was used. J. M. Stewart, Technical Report TR-446 of Computer Science Center, University of Maryland (1976).

[18] Crystallographic coordinates have been deposited with the Cambridge Crystallographic Data Centre.