Synthesis of Alanyl-1-aminoethanesulfonic Acid[1)]

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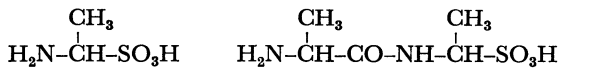
For the purpose of the verification of an expectation that the structural analogs of D-alanyl-D-alanine which participates in the biosynthesis of cell wall peptides might inhibit the growth of microorganisms, alanyl-1-aminoethanesulfonic acid was synthesized by the mixed anhydride method. Four diastereomers were separated and their absolute configurations were deduced on the basis of X-ray analysis. None of these dipeptide analogs and their benzyloxycarbonyl or *t*-butoxycarbonyl derivatives showed appreciable activity against *E. coli*.

α-Amino sulfonic acids can often act as antagonists of α-amino carboxylic acids. In 1941, McIlwain[2)] prepared several α-amino sulfonic acids which were found to possess antimicrobial activities. Thereafter, another author reported antivial activity of some amino sulfonic acids.[3)] The analogy between amino carboxylic acid and amino sulfonic acid in biological effect was also proved in meaty taste of γ-sulfonic acid analog of L-glutamic acid.[4)] In this line, an activity as antagonist will be expected in a structural analog of a biologically significant peptide where one of the component amino acids is replaced with the corresponding amino sulfonic acid.

From studies on the biosynthesis of bacterial cell wall,[5)] it has been revealed that D-alanyl-D-alanine is incorporated as a dipeptide unit into a peptidoglycan precursor in an early step and the C-terminal D-alanine residue finally splits off for completion of the network structure of the peptidoglycan through transpeptidation. If an amino sulfonic acid peptide acts as an antagonist of D-alanyl-D-alanine, it may possibly inhibit either the incorporation of the dipeptide or the formation of the cross linkage of the peptide chain.[6)]

On the basis of above consideration, we planned to prepare the compounds in which one alanine residue of the dipeptide is replaced with 1-aminoethanesulfonic acid (Aes) (**1**).[7)] In this paper, the synthesis and separation of four stereoisomers of alanyl-1-aminoethanesulfonic acid (**4** and **6**) are described.



1 **4, 6**

Although Frankel and Moses[8)] reported the preparation of some (*N*-benzyloxycarbonylglycyl)amino sulfonic acids, problems remained to be solved on the stereochemistry of amino sulfonic acids. Mild conditions for deprotection also seemed to have to be investigated.

Results and Discussion

As a preliminary experiment, benzyloxycarbonyl derivative of 1-aminoethanesulfonic acid (**1**) was prepared in order to examine the chemical properties of acylated α-amino sulfonic acid and to investigate the method of deprotection. 1-(Benzyloxycarbonylamino)-ethanesulfonic acid (**2**) was isolated as crystalline anilinium salt which was readily characterized.[9)] Isolation of this sulfonic acid (**2**) as its sodium salt was not preferable since removal of inorganic salt from it became very difficult because of the similar solubilities in water. When benzyloxycarbonyl derivative was hydrogenolyzed in the presence of palladium catalyst, free 1-aminoethanesulfonic acid (**1**) was obtained, though in a low yield (12%). This indicates that the amino sulfonic acid structure can survive more or less through hydrogenolysis depending on the reaction conditions. Thus, the benzyloxycarbonyl group could be used as appropriate protecting group for the synthesis of the amino sulfonic acid peptides.

In a similar manner to the usual peptide synthesis, benzyloxycarbonyl-D-alanine was coupled with sodium salt of 1-aminoethanesulfonic acid (**1**) using ethyl chloroformate to afford benzyloxycarbonyl-D-alanyl-1-aminoethanesulfonic acid (**3**), which was isolated as crystalline anilinium salt. Since racemic **1** was employed as the starting material, the product (**3**) should be a mixture of diastereomers, *i.e.*, benzyloxycarbonyl-D-alanyl-D-1-aminoethanesulfonic acid and its D-L isomer.[10)] However, this product showed no sign of heterogenity either on TLC or on recrystallization.

Catalytic hydrogenolysis of **3** gave free D-alanyl-1-aminoethanesulfonic acid (**4**) by removal of the benzyloxycarbonyl group in a good yield. This product (**4**) showed two ninhydrin positive spots on TLC or paper chromatography (PC). Both components (**4a** and **4b**) which were separated each other by preparative PC had the same molecular formula and thus could be assigned to be the diastereoisomers of D-alanyl-1-aminoethanesulfonic acid, though the configurations of the amino sulfonic acid residues were not clarified at this stage.

In order to confirm this result, the same reactions were carried out starting from benzyloxycarbonyl-L-alanine. Thus, deprotection of benzyloxycarbonyl-L-alanyl-1-aminoethanesulfonic acid (**5**) followed by separation with preparative PC afforded the diastereomers of L-alanyl-1-aminoethanesulfonic acid (**6a** and **6b**). Purities of **6a** and **6b** were well monitored by means of NMR spectra, since slight but distinct differences of chemical shifts between the two compounds are observed at the protons of L-alanine residues (see experimental section). The NMR spectrum of the deprotection product (**6**) before separation corresponds to that expected for a 1 : 1 mixture of **6a** and **6b**.

X-Ray analysis of **6a**[11)] established the *R* configuration for the asymmetric carbon atom of the 1-aminoethanesulfonic acid residue in this diastereomeric form by taking account of the *S* configuration of the L-alanine residue in the same molecule as reference. If D, L re-

Table 1. Physical constants and stereochemistry of diastereomers of alanyl-1-aminoethanesulfonic acid

	Mp (dec) °C	Rf on PC[a]	$[\alpha]_D^{26}$ [b]	Configuration of	
				Ala	Aes[c]
4a	230	0.23	+134°	*R* (D)	*S* (D)[d]
4b	213	0.27	−175°	*R* (D)	*R* (L)
6a	234	0.23	−128°	*S* (L)	*R*[e] (L)
6b	213	0.27	+171°	*S* (L)	*S* (D)

a) Toyo No. 51 filter paper, 1-butanol–acetic acid–water 4:1:2. b) *c* 0.5 in 0.5 M HCl. c) Aes: 1-aminoethanesulfonic acid. d) See Ref. 10 in the text. e) Determined by X-ray analysis.

presentation for amino acid is applied to the configuration of aminoethanesulfonic acid, **6a** should be termed as L-alanyl-L-1-aminoethanesulfonic acid. Accordingly, the stereostructures of the other three "dipeptides" were unequivocally assigned by comparison of their physical constants each other. These results are summarized in Table 1.

Consequently, we could thus obtain two pairs of optically active alanyl-1-aminoethanesulfonic acids and determine their absolute configurations. Furthermore, it was also revealed that the configuration of the α-carbon atom in an α-amino sulfonic acid residue is retained stable if its amino group is acylated, although it is generally accepted that α-amino sulfonic acids are unstable, thus being readily dissociated to the parent aldehydes, ammonia and bisulfite in aqueous solution. There has been reported only one case of securing an optically active α-amino sulfonic acid derivative by Neelakantan so far.[12] On reaction of ephedrine with sodium hydrogen sulfite adduct of benzaldehyde, he obtained one of the two possible diastereomers of the amino sulfonic acid derivative and another isomer could not be obtained which was immediately transformed *via* intramolecular cyclization. Therefore, our result in this investigation would be the first and significant example in the determination of stereochemistry of α-amino sulfonic acid.

For further synthesis of more complex peptide analogs containing amino sulfonic acid, applicability of other protecting groups should be examined. *t*-Butoxycarbonyl group was shown to be satisfactorily useful. Thus, *t*-butoxycarbonyl-D and L-alanines were condensed with 1-aminoethanesulfonic acid (**1**) to yield *t*-butoxycarbonyl-D and L-alanyl-1-aminoethanesulfonic acids (**7** and **8**) respectively. Removal of *t*-butoxycarbonyl group was carried out by means of dry hydrogen chloride in ethyl acetate. From the anilinium salt of **8** was obtained free L-alanyl-1-aminoethanesulfonic acid (**6**), which was identified by means of TLC and IR spectra with the sample obtained above by hydrogenolysis of benzyloxycarbonyl-L-alanyl-1-aminoethanesulfonic acid (**5**). When the anilinium salt of the benzyloxycarbonyl derivative (**5**) was treated with anhydrous hydrogen bromide in acetic acid, the protecting group was readily removed and the product was identified with **6** by TLC. However, separation of pure substance from contaminating anilinium bromide was hardly effected.

Finally, antimicrobial activity was tested for the four diastereomers of alanyl-1-aminoethanesulfonic acid (**4a**, **4b**, **6a**, and **6b**) and sodium salts of benzyloxycarbonyl- as well as *t*-butoxycarbonyl-D and L-alanyl-1-aminoethanesulfonic acids (**3**, **5**, **7**, and **8**). Unfortunately, none of them showed appreciable activity against *E. coli* in a modified Henderson-Snell medium free from alanine even in high concentrations of 500—1000 μg/ml.

Experimental[13]

1-Aminoethanesulfonic Acid (1). This compound was prepared through the reaction of acetaldehyde either with ammonium sulfite[2a] or with sodium hydrogen sulfite and ammonia;[14] mp 171—172 °C dec. Use of the former reagent was more convenient though the yield was somewhat lower than the latter. The product was used to the following coupling reaction without recrystallization because of its lability toward decomposition on heating or even on standing at room temperature in an aqueous solution.

1-(Benzyloxycarbonylamino)ethanesulfonic Acid (2) Anilinium Salt. To an ice-cooled solution of **1** (1.25 g, 10 mmol) and Na_2CO_3 (1.17 g, 11 mmol) in water (10 ml), there was added benzyloxycarbonyl chloride (2.1 g, 12 mmol) dropwise with stirring. Stirring was continued at room temperature overnight and excess of the chloride was extracted with ether. After the aqueous layer was concentrated *in vacuo*, aniline (0.93 g, 10 mmol) was added and the mixture was adjusted to pH 6 with 6 M HCl under ice cooling. The crystalline anilinium salt of **2** was collected by filtration; yield 1.35 g (38%). Recrystallization was effected from methanol–ether; mp 164—166 °C dec.

Found: C, 54.34; H, 5.68; N, 7.91; S, 9.09%. Calcd for $C_{16}H_{20}O_5N_2S$: C, 54.53; H, 5.72; N, 7.95; S, 9.10%.

Hydrogenolytic Deprotection of 2. The anilinium salt of **2** (0.70 g, 2 mmol) was dissolved in methanol (20 ml), and hydrogenolyzed in the presence of Pd black and acetic acid (0.12 ml, 2 mmol) at room temperature. After 3 h, the catalyst and the white precipitates formed were filtered and the latter were extracted with water at 40 °C. The aqueous extract was concentrated *in vacuo* and allowed to stand in a refrigerator to form colorless crystals; yield, 30 mg (12%); mp 170—172 °C dec. The IR spectrum of this product was completely identical with that of **1**.

Benzyloxycarbonyl-D-alanyl-DL-1-aminoethanesulfonic Acid (3) Anilinium Salt. Ethyl chloroformate (0.24 ml, 2.5 mmol) was added to a solution of benzyloxycarbonyl-D-alanine (0.53 g, 2.4 mmol) and *N*-methylmorpholine (0.29 ml, 2.6 mmol) in anhydrous tetrahydrofuran (5 ml) at −13 °C with stirring. After 10 min, a solution of **1** (0.45 g, 3.6 mmol) in 2 M aqueous NaOH (1.8 ml) was added dropwise during 5 min, while the mixture was stirred at −13 °C. Stirring was continued at this temperature for further 3 h. After tetrahydrofuran was evaporated *in vacuo*, the mixture was diluted with water, treated with aniline (0.35 ml, 3.8 mmol) and adjusted to pH 6 with 2 M HCl under ice cooling. The crystals of anilinium salt of **3** were filtered; yield, 0.47 g (46%). Recrystallization was effected from methanol–ether; mp 201—204 °C dec.

Found: C, 53.88; H, 5.94; N, 9.93; S, 7.67%. Calcd for $C_{19}H_{25}O_6N_3S$: C, 53.88; H, 5.95; N, 9.92; S, 7.57%.

D-Alanyl-DL-1-aminoethanesulfonic Acid (4). The anilinium salt of **3** (1.27 g, 3.0 mmol) was dissolved in methanol and hydrogenolyzed in the presence of Pd black at room temperature. After the catalyst was filtered off and solvent

was evaporated *in vacuo*, the residue was recrystallized from water–methanol–ether; yield, 0.46 g (77%); mp 220—224 °C dec; R_f 0.23, 0.27 (Toyo No. 51 filter paper, 1-butanol–acetic acid–water 4: 1: 2).

Separation of D-*Alanyl*-D *and* L-*1-aminoethanesulfonic Acids (**4a** and **4b**).* The mixture (400 mg) of the diastereomers (**4**) obtained above was dissolved in a small amount of water and subjected to a preparative PC on Toyo No. 50 filter paper (40×40 cm, 4 pieces) by developing twice with 1-butanol–acetic acid–water (4: 1: 2). The three portions of a ninhydrin positive band were extracted with water separately. From each extract, pure **4a** (75 mg), pure **4b** (40 mg), and a mixture of them (105 mg) were obtained respectively by crystallization from water–methanol–ether.

D-*Alanyl*-D-*1-aminoethanesulfonic Acid (**4a**)*: Physical constants; see Table 1.

Found: C, 27.70; H, 6.62; N, 13.07; S, 14.85%. Calcd for $C_5H_{12}O_4N_2S \cdot H_2O$: C, 28.03; H, 6.59; N, 13.08; S, 14.97%.

D-*Alanyl*-L-*1-aminoethanesulfonic Acid (**4b**)*: Physical constants; see Table 1.

Found: C, 29.91; H, 6.11; N, 13.98; S, 15.86%. Calcd for $C_5H_{12}O_4N_2S \cdot 1/4H_2O$: C, 29.92; H, 6.28; N, 13.96; S, 15.97%.

Benzyloxycarbonyl-L-*alanyl*-DL-*1-aminoethanesulfonic Acid (**5**) Anilinium Salt.* Ethyl chloroformate (1.60 ml, 17 mmol) was added to a solution of benzyloxycarbonyl-L-alanine (3.40 g, 15 mmol) and *N*-methylmorpholine (1.80 ml, 16 mmol) in anhydrous tetrahydrofuran (35 ml) at −9 °C with stirring. After 8 min, there was added a solution of **1** (2.50 g, 20 mmol) in 1.5 M aqueous NaOH (13 ml) with stirring at −9 °C. The mixture was stirred at room temperature for 3 h. The product was converted into its anilinium salt as described above for **3** and recrystallized from methanol–ether; yield 1.90 g (30%); mp 193—194 °C dec. Its IR spectrum was identical with that of the anilinium salt of **3**.

Found: C, 53.51; H, 5.82; N, 9.92; S, 7.56%. Calcd for $C_{19}H_{25}O_6N_3S$: C, 53.88; H, 5.95; N, 9.92; S, 7.57%.

L-*Alanyl*-DL-*1-aminoethanesulfonic Acid (**6**).* The anilinium salt of **5** (1.27 g, 3.0 mmol) was hydrogenolyzed as described above for **4** and the product was recrystallized from water–methanol–ether; yield 0.58 g (97%); mp 224—226 °C dec. NMR[15]: δ 1.51 (3H, d, J=7, CH_3 of Aes), 1.55 and 1.57 (each 1.5H, d, J=7, CH_3 of Ala), 4.11 and 4.12 (each 0.5H, q, J=7, CH of Ala), 5.02 (1H, q, J=7, CH of Aes). The IR spectrum was identical with that of **4**.

Found: C, 29.99; H, 6.23; N, 13.88; S, 16.10%. Calcd for $C_5H_{12}O_4N_2S \cdot 1/4H_2O$: C, 29.92; H, 6.28; N, 13.96; S, 15.97%.

Separation of L-*Alanyl*-L *and* D-*1-aminoethanesulfonic Acids (**6a** and **6b**).* The mixture (400 mg) of the diastereomers obtained above was subjected to preparative PC as described for **4a** and **4b**, affording pure **6a** (130 mg), pure **6b** (130 mg), and a mixture of them (90 mg).

L-*Alanyl*-L-*1-aminoethanesulfonic Acid (**6a**)*: Physical constants; see Table 1. NMR[15]: δ 1.51 (3H, d, J=7, CH_3 of Aes), 1.57 (3H, d, J=7, CH_3 of Ala), 4.12 (1H, q, J=7, CH of Ala), 5.02 (1H, q, J=7, CH of Aes).

Found: C, 27.88; H, 6.66; N, 13.01; S, 14.72%. Calcd for $C_5H_{12}O_4N_2S \cdot H_2O$: C, 28.03; H, 6.59; N, 13.08; S, 14.97%.

L-*Alanyl*-D-*1-aminoethanesulfonic Acid (**6b**)*: Physical constants; see Table 1. NMR[15]: δ 1.51 (3H, d, J=7, CH_3 of Aes), 1.55 (3H, d, J=7, CH_3 of Ala), 4.11 (1H, q, J=7, CH of Ala), 5.02 (1H, q, J=7, CH of Aes).

Found: C, 29.95; H, 6.24; N, 14.11; S, 15.97%. Calcd for $C_5H_{12}O_4N_2S \cdot 1/4H_2O$: C, 29.92; H, 6.28; N, 13.96; S, 15.97%.

t-*Butoxycarbonyl*-D-*alanyl*-DL-*1-aminoethanesulfonic Acid (**7**).* Ethyl chloroformate (0.53 ml, 5.5 mmol) was added to a solution of *t*-butoxycarbonyl-D-alanine (0.95 g, 5.0 mmol) and *N*-methylmorpholine (0.60 ml, 5.5 mmol) in anhydrous tetrahydrofuran (15 ml) at −8 °C with stirring. After 10 min, there was added a solution of **1** (0.95 g, 7.6 mmol) in 1 M aqueous NaOH (7.5 ml) dropwise with stirring at −8 °C. Stirring was continued at this temperature for 1 h and at room temperature for further 3 h. Tetrahydrofuran was removed *in vacuo* and the product was isolated as its anilinium salt as described for **3** and recrystallized from methanol–ether; yield, 0.84 g (43%); mp 163 °C dec. An analytical sample was further recrystallized from the same solvents; mp 179—181 °C dec.

Found: C, 49.09; H, 7.02; N, 10.77; S, 8.22%. Calcd for $C_{16}H_{27}O_6N_3S$: C, 49.34; H, 6.99; N, 10.79; S, 8.23%.

t-*Butoxycarbonyl*-L-*alanyl*-DL-*1-aminoethanesulfonic Acid (**8**).* This compound was prepared as described above for **7** by using *t*-butoxycarbonyl-L-alanine (0.95 g, 5.0 mmol) in place of *t*-butoxycarbonyl-D-alanine. The product was isolated as its anilinium salt; yield, 0.50 g (26%); mp 162—166 °C dec. Its IR spectrum was identical with that of the anilinium salt of **7**.

*Deprotection of **8**.* To a suspension of anilinium salt of **8** (100 mg, 0.26 mmol) in ethyl acetate (3 ml), there was added a saturated solution of dry hydrogen chloride in ethyl acetate (2 ml). While the mixture was stirred at room temperature for 90 min, the initial crystals dissolved and the product separated out. The latter was collected by filtration and recrystallized from water–methanol–ether; yield, 20 mg (40%); mp 220—223 °C dec. Its IR spectrum was identical with that of **6**.

*Sodium Salts of **3**, **5**, **7**, and **8**.* These compounds were prepared by dissolving the corresponding anilinium salt in water containing one equivalent of NaOH, followed by extraction with ether and evaporation of the aqueous phases *in vacuo*.

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