Probiotics: Antistaphylococcal Activity of 4-Aminocyclohexanecarboxylic Acid, Aminobenzoic Acid, and Their Derivatives and Structure–Activity Relationships

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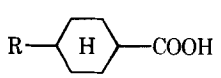
Abstract □ Derivatives of 4-aminocyclohexanecarboxylic acid and aminobenzoic acid were prepared and tested against experimentally induced *Staphylococcus aureus* infections in mice. *cis*- and *trans*-Guanidinocyclohexanecarboxylic acid, *trans*-4-aminomethylcyclohexanecarboxylic acid, *trans*-4-guanidinomethylcyclohexanecarboxylic acid, 4-aminomethylbenzoic acid, 4-guanidinomethylbenzoic acid, 4-(2-aminoethyl)benzoic acid, 4-(2-guanidinoethyl)benzoic acid, and most of the ω-aminoacyl-4-aminobenzoic acids possessed antistaphylococcal activity. The R_m values of these compounds were determined by a reversed-phase TLC system. Experimental R_m values of several additional series of probiotics (ω-amino acids, ω-aminoacyl-L-histidines, ω-guanidino acids, ω-guanidinoacyl-L-histidines, and ω-aminoalkanesulfonic acids) were also obtained. A regular relationship was observed between the constitution of the mobile phases and the R_m values; the optimal mobile phase was 70% acetone in water on silica gel G TLC plates. In all series, relationships were obtained between the biological response (antistaphylococcal activity per millimole) and the R_m values, with an increased R_m value paralleling an increased antistaphylococcal activity.

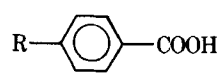
Keyphrases □ 4-Aminocyclohexanecarboxylic acid and derivatives—synthesized, evaluated for antistaphylococcal activity *in vivo*, mice □ Aminobenzoic acid and derivatives—synthesized, evaluated for antistaphylococcal activity *in vivo*, mice □ Structure–activity relationships—4-aminocyclohexanecarboxylic acid, aminobenzoic acid, and various derivatives evaluated for antistaphylococcal activity □ Antistaphylococcal activity—4-aminocyclohexanecarboxylic acid, aminobenzoic acid, and various derivatives evaluated

The term probiotics was proposed to designate compounds that build resistance to infection in the host but do not inhibit the growth of microorganisms *in vitro* (1). Investigations of probiotics (1–5) provided several series of compounds active against experimentally induced *Staphylococcus aureus* infections in mice. A relationship was indicated between the degree of protection afforded by the compounds against staphylococcal infections and the distance separating the functional groups, *i.e.*, between the ω-amino and carboxyl groups of ω-amino acids (2) and their dipeptides (2–4), between ω-guanidino and carboxyl groups of ω-guanidino acids and their dipeptides (1), and between ω-amino, ω-guanidino, and sulfonyl groups of ω-amino- and ω-guanidinoalkanesulfonic acids (5). The relationship between antifibrinolytic activity and the distance separating functional groups of ω-amino acids and related compounds was demonstrated (6–13). A possible relationship between the biological response and R_m values of probiotics also was reported (5).

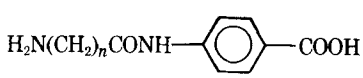
The present report describes the synthesis and antistaphylococcal activity of derivatives of 4-aminocyclohexanecarboxylic acid (I*a*), aminobenzoic acid (II*a*), and ω-aminoacyl-4-aminobenzoic acids (III*a*–III*e*) and discusses the relationship between biological response of probiotics and R_m values.



I*a*: R = NH_2
I*b*: R = $NHC(=NH)NH_2$
I*c*: R = CH_2NH_2
I*d*: R = $CH_2NHC(=NH)NH_2$



II*a*: R = NH_2
II*b*: R = $NHC(=NH)NH_2$
II*c*: R = CH_2NH_2
II*d*: R = $CH_2NHC(=NH)NH_2$
II*e*: R = $(CH_2)_2NH_2$
II*f*: R = $(CH_2)_2NHC(=NH)NH_2$



III*a*: *n* = 1
III*b*: *n* = 2
III*c*: *n* = 3
III*d*: *n* = 4
III*e*: *n* = 5

BACKGROUND

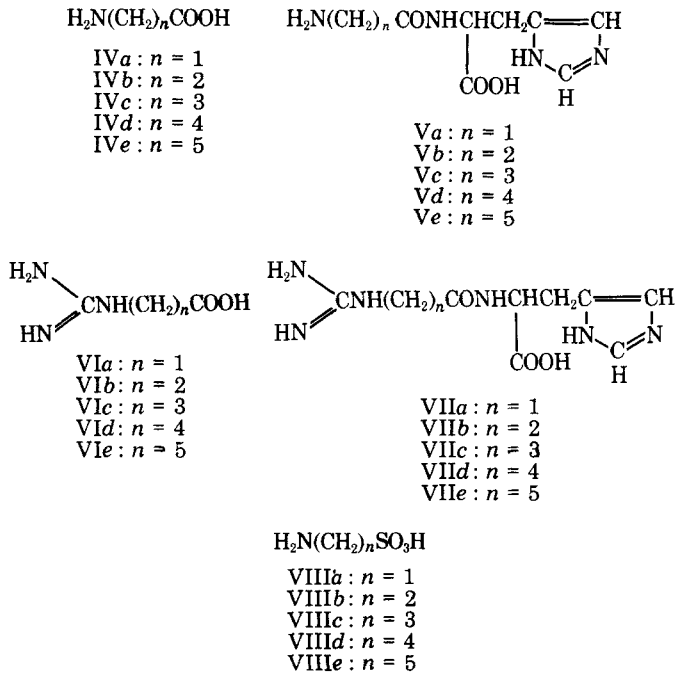
Compound I*a* was prepared by a modification of the method described by Pearlman (14) by hydrogenation of II*a* with hydrogen in the presence of 10% rhodium–0.1% palladium-on-carbon. Compound I*a* was also prepared by the method of Takagi *et al.* (15–18), using platinum dioxide in the presence of hydrogen gas. Compounds I*b*, I*d*, II*d*, and II*f* were prepared from I*a*, I*c*, II*c*, and II*e*, respectively, by a modification of the method reported by Morrison *et al.* (19). Compound II*b* was prepared from II*a* by a method described by Beyerman and Bontekoe (20). Compounds II*c* and II*e* were prepared from 4-bromomethylbenzoic acid and 4-(2-bromoethyl)benzoic acid, respectively, by treatment with concentrated ammonium hydroxide. Compounds III*a*–III*e* were prepared from the appropriate benzyloxycarbonyl-ω-amino acid by a reported method (2) that was modified using several literature methods (21–23).

To investigate a possible relationship between biological response and physicochemical character, R_m values of probiotics also were determined. The lipophilic–hydrophilic balance of a compound, which can be expressed by a partition coefficient, is critical for drug transport. The theoretical relationship between the partition coefficient, P, and R_f values was deduced (24–26) for liquid–liquid partition chromatography, and this relationship was simplified (27) to:

$$R_m = \log\left(\frac{1}{R_f} - 1\right) \qquad \text{(Eq. 1)}$$

Since R_m values are free-energy-based constants related to the π values of the Hansch approach, it is possible in principle to correlate the biological action of a drug with R_m values (28–32).

The optimal mobile phase constitution was first determined using silica gel G plates impregnated with paraffin oil white for ω-amino acids (IV*a*–IV*e*) and ω-aminoacyl-L-histidines (V*a*–V*e*), which possess antistaphylococcal activity (2). The optimal mobile phase was 70% acetone in water. The R_m values of derivatives of I*a* and II*a*, III*a*–III*e*, and additional series of probiotics, ω-guanidino acids (VI*a*–VI*e*), ω-guanidinoacyl-L-histidines (VII*a*–VII*e*), and ω-aminoalkanesulfonic acids (VIII*a*–VIII*e*), were then determined, using the optimal mobile phase, to investigate structure–activity relationships.



EXPERIMENTAL[1]

10% Rhodium–0.1% Palladium-on-Carbon—This catalyst was prepared by the method of Pearlman (14), using a mixture of 40 g of rhodium chloride trihydrate[2], 0.26 g of palladium chloride[2], 13.7 g of carbon[3], and 200 ml of water. The yield was 15.8 g.

***cis*- and *trans*-4-Aminocyclohexanecarboxylic Acid (Ia)**—*Method A*—A mixture of 27.4 g (0.200 mole) of IIa[4], 200 ml of water, and 4 g of 10% rhodium–0.1% palladium-on-carbon was placed in an autoclave[5] and hydrogenated at 3.52 kg/cm^2. When 0.26 mole of hydrogen gas was absorbed, the mixture was filtered and concentrated *in vacuo* until crystals started to form. Then the mixture was diluted with 200 ml of dimethylformamide and cooled to 5°. The precipitate was filtered, washed first with dimethylformamide and then cold methanol, and dried. The yield was 5.3 g (18.5%) [lit. (14) yield 68–71%], mp 271–275° dec. [lit. (14) mp 292–296°].

Method B—The compound was also prepared by the method of Takagi *et al.* (15–18) by pressure hydrogenation for 6 days in an autoclave[5] of a mixture of 20.0 g (0.146 mole) of IIa, 300 ml of water, and 2.0 g (0.085 mole) of platinum dioxide[3]. The yield was 6.6 g (32.0%), mp 269–270° dec. [lit. (14) mp 292–296°].

***cis*- and *trans*-4-Guanidinocyclohexanecarboxylic Acid (Ib)**—A mixture of 2.9 g (0.021 mole) of Ia and 4.5 g (0.053 mole) of *S*-methylisothiourea hemisulfate[6] in 30 ml of concentrated ammonium hydroxide was refluxed until all reactants were dissolved. After filtration, the solution was concentrated *in vacuo*.

The resulting syrup was diluted with pH 2.2 pyridine–formic acid buffer for purification by ion-exchange chromatography[7]. The sample was eluted with 250 ml of water, 250 ml of 0.5 *M* pyridine, 450 ml of 2 *M* pyridine, and then 200 ml of 2 *M* pyridine–2 *N* NH_4OH (50:50 v/v). Fractions containing Ib were pooled and concentrated *in vacuo* and crystallized from hot water. The yield was 0.6 g (20.0%), mp > 370°.

Anal.—Calc. for $C_8H_{15}N_3O_2$: C, 51.88; H, 8.16; N, 22.69. Found: C, 51.76; H, 8.10; N, 22.55.

***trans*-4-Guanidinomethylcyclohexanecarboxylic Acid (Id)**—A mixture of 2.0 g (0.013 mole) of Ic[8], 2.36 g (0.017 mole) of *S*-methylisothiourea hemisulfate[6], and 10 ml of concentrated ammonium hydroxide was treated in the same way as for Ib. The yield was 2.0 g (78.5%), mp 353–354° dec. [lit. (33) mp 349° dec.].

4-Guanidinomethylbenzoic Acid (IId)—A mixture of 1.43 g (0.0095 mole) of IIc, 2.1 g (0.0145 mole) of *S*-methylisothiourea hemisulfate[6], and 25 ml of concentrated ammonium hydroxide was allowed to react in the same way as already described. The yield was 1.32 g (72.0%), mp 303–304° dec.

Anal.—Calc. for $C_9H_{11}N_3O_2 \cdot H_2O$: C, 51.18; H, 6.20; N, 19.89. Found: C, 51.66; H, 6.10; N, 19.89.

4-(2-Guanidinoethyl)benzoic Acid (IIf)—A mixture of 5.0 g (0.030 mole) of IIe, 5.95 g (0.046 mole) of *S*-methylisothiourea hemisulfate[6], and 40 ml of concentrated ammonium hydroxide was allowed to react in the same way as for IId. The yield was 5.7 g (91.0%), mp 328–330° dec.

Anal.—Calc. for $C_{10}H_{13}N_3O_2$: C, 57.95; H, 6.32; N, 20.28. Found: C, 57.80; H, 6.20; N, 20.15.

4-Guanidinobenzoic Acid (IIb)—By using the method of Beyerman and Bontekoe (20), thioureidobenzoic acid was prepared from 15.8 g (0.115 mole) of IIa and 12.1 g (0.160 mole) of ammonium thiocyanate in 42 ml of concentrated hydrochloric acid and 75 ml of water. Thioureidobenzoic acid (10.65 g, 0.057 mole) and 8.1 g (0.057 mole) of methyl iodide in 82 ml of absolute ethanol[9] gave *S*-methylisoureidobenzoic acid. Compound IIb was obtained by refluxing 10.0 g (0.030 mole) of *S*-methylisoureidobenzoic acid with concentrated ammonium hydroxide. The yield was 4.26 g (62.0%), mp 300–302° dec. [lit. (20) mp ~310° for the free base and 268° dec. for the hydrochloride].

4-Aminomethylbenzoic Acid (IIc)—4-Bromomethylbenzoic acid[10] (14.0 g, 0.070 mole) was dissolved in 2100 ml of concentrated ammonium hydroxide and allowed to stand at room temperature for 2 weeks. The solution was concentrated to dryness *in vacuo*, and the residue was crystallized from water after the addition of absolute ethanol[9]–ether. The crystals were then filtered and subjected to ion-exchange chromatography using a 3 × 35-cm column[7] and the same eluents as in the preparation of Ib.

The elution of IIc was detected by the ninhydrin reaction and appeared in 500–700 ml of the effluent. These fractions were concentrated to dryness *in vacuo*. The residue was crystallized from water after the addition of absolute ethanol[9]–ether; yield, 6.92 g (70.7%); mp 351–353° dec. [lit. (34) mp 346–348° dec.].

4-(2-Aminoethyl)benzoic Acid (IIe)—4-(2-Bromoethyl)benzoic acid[10] (5.0 g, 0.0218 mole) and 675 ml of concentrated ammonium hydroxide were treated in the same way as for IIc, except that the ion-exchange purification was omitted. The residue, after concentration and the addition of absolute ethanol[9]–ether, was crystallized from water. The yield was 1.65 g (46.0%), mp >370°.

Anal.—Calc. for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.32; H, 6.60; N, 8.49.

4-(Glycylamino)benzoic Acid (IIIa)—To a solution of 10.5 g (0.050 mole) of benzyloxycarbonylglycine[11] in 250 ml of dichloromethane was added 7.0 ml of triethylamine. After the resulting solution had been chilled to −5°, 4.8 ml of ethyl chloroformate was added and the mixture was kept at this temperature for 10 min. To this solution was added rapidly a solution of ethyl 4-aminobenzoate[12] (8.3 g, 0.050 mole) in 100 ml of dichloromethane, which had been chilled to 0°.

The resulting mixture was stored at room temperature for 3 days. It was then washed with 200 ml of water and 200 ml of 1 *N* $NaHCO_3$, dried over sodium sulfate, and concentrated to a syrup. The concentrate was dissolved in 100 ml of methanol, and insoluble material (unreacted benzyloxycarbonylglycine) was filtered off. To the filtrate was added 50 ml of 1 *N* NaOH, and the mixture was kept at room temperature for 3 hr. The reaction mixture was adjusted to pH 5 with 4 *N* H_2SO_4 and concentrated to dryness *in vacuo*. The residue was extracted with two portions of 50 ml each of hot absolute ethanol[9], and 50 ml of water was added to the extract.

After the addition of 1.0 g of 10% palladium-on-carbon, the mixture was hydrogenated in an autoclave at atmospheric pressure and room temperature for 6 hr. The reaction mixture was then filtered and concentrated *in vacuo*. Ion-exchange chromatography, with a 2.0 × 30-cm column[7], was used to purify the product. The fractions containing IIIa were pooled, concentrated *in vacuo*, and then treated with absolute ethanol[9] to obtain white needle crystals. The yield was 1.55 g (15.0%), mp 237–238° dec.

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[1] IR spectra (potassium bromide) were taken of all compounds with a Perkin-Elmer IR spectrophotometer, model 337, and were in accord with the assigned structures. Elemental analyses were made of all compounds by Galbraith Laboratories, Inc., Knoxville, Tenn., but analyses and melting points are reported only for new compounds. Solvents and chemicals, except where indicated, were purchased from Fisher Scientific Co. and Matheson, Coleman and Bell.

[2] Alfa Products.

[3] Darco G-60.

[4] Aldrich Chemical Co.

[5] Parr Instrument Co., series 450.

[6] Sigma Chemical Co.

[7] Amberlite CG 120, 200–400 mesh, pyridine form.

[8] A gift from Daiichi Seiyaku Co., Ltd., Tokyo, Japan.

[9] Commercial Solvents Corp.

[10] ICN Pharmaceuticals, Inc. (K&K Laboratories).

[11] Carbobenzoxyglycine, ICN Pharmaceuticals, Inc.

[12] Eastman Kodak Co.

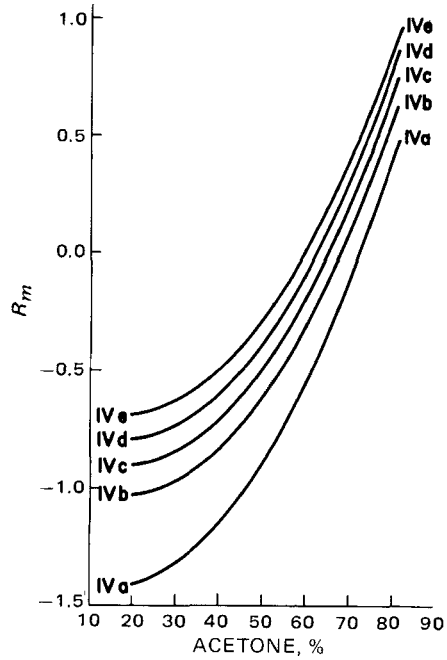

Figure 1—*Relationship between* R_m *values of ω-amino acids (series IV) and percent acetone in mobile phase.*

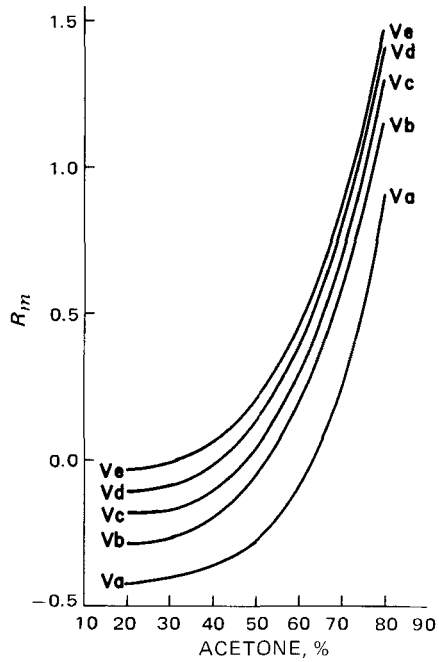

Figure 2—*Relationship between* R_m *values of ω-aminoacyl-L-histidines (series V) and percent acetone in mobile phase.*

Anal.—Calc. for $C_9H_{10}N_2O_3 \cdot H_2O$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.72; H, 5.48; N, 13.20.

After hydrolysis (6 *N* HCl, 105° for 24 hr), III*a* gave glycine and II*a* as confirmed on TLC.

4-(β-Alanylamino)benzoic Acid (III*b*)—Benzyloxycarbonyl-β-alanine[13] (4.4 g, 0.020 mole) and 3.3 g (0.020 mole) of ethyl 4-aminobenzoate[12] were treated in the same way as for III*a*. The yield was 2.1 g (47.4%), mp 256–257° dec.

Anal.—Calc. for $C_{10}H_{12}N_2O_3 \cdot \frac{1}{2}H_2O$: C, 55.29; H, 6.03; N, 12.90. Found: C, 55.04; H, 6.17; N, 12.80.

Acid hydrolysis gave β-alanine and II*a*.

4-(4-Aminobutyrylamino)benzoic Acid (III*c*)—4-(Benzyloxycarbonylamino)butyric acid[14] (12.0 g, 0.050 mole) and 8.3 g (0.050 mole) of ethyl 4-aminobenzoate[12] were treated in the same manner as for III*a*. The yield was 5.2 g (44.2%), mp 214–215° dec.

Anal.—Calc. for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.61. Found: C, 59.20; H, 6.50; N, 12.43.

Acid hydrolysis gave 4-aminobutyric acid and II*a*.

4-(5-Aminovalerylamino)benzoic Acid (III*d*)—5-(Benzyloxycarbonylamino)valeric acid[15] (12.5 g, 0.050 mole) and 8.3 g (0.050 mole) of ethyl 4-aminobenzoate[12] were treated in the same manner as for III*a*. The yield was 5.0 g (40.0%), mp 203–204°.

Anal.—Calc. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.82; H, 6.96; N, 11.73.

Acid hydrolysis gave 5-aminovaleric acid and II*a*.

4-(6-Aminohexanoylamino)benzoic Acid (III*e*)—6-(Benzyloxycarbonylamino)hexanoic acid[16] (2.65 g, 0.010 mole) and 1.65 g (0.010 mole) of ethyl 4-aminobenzoate were treated in the same way as for III*a*. The yield was 1.20 g (45.4%), mp 258–259° dec.

Anal.—Calc. for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.19; H, 7.32; N, 11.05.

Acid hydrolysis gave 6-aminohexanoic acid and II*a*.

Reversed-Phase TLC—The R_m values were obtained by the method described by Boyce and Milborrow (28) using silica gel G TLC plates. Chromatography was carried out on glass plates[17] (10 × 20 cm) coated with a 250-μm layer of silica gel G. After activation at 105° for 10 min, the plates were impregnated by allowing a 5% (v/v) solution of paraffin oil white in hexane to cover the plate; the solvent was evaporated at 40°.

Optimal Solvent—The optimal mobile phase composition was determined using the mobile phases of 80, 70, 60, 50, 40, and 20% (v/v) acetone in water. Solutions that contained 1% of IV*a*–IV*e*, V*a*–V*e*, and L-alanine dissolved in 10% (w/v) 2-propanol were spotted and then developed[18] with each different mobile phase. The spots were located by the ninhydrin reaction. The relationship between R_m values of series IV and V and the composition of the mobile phases is shown in Figs. 1 and 2. The optimal solvent ratio, which produced maximum increments of R_m values (28, 29), was obtained from the most linear region of the curves: 70% (v/v) acetone in water.

Determination of R_m Values with Optimal Solvent System—With the optimal composition of the mobile phase, TLC plates were developed after spotting with series I–VIII; L-alanine and L-arginine were used as standards. The spots were located by the ninhydrin reaction, the Sakaguchi reaction, or iodine treatment. Figure 3 summarizes the relationship between biological response and R_m values of series IV–VIII.

Biological Testing Procedure—*In vivo* antistaphylococcal activity was determined by the method described previously (1–5), except that BDF mice, 9–13 weeks old and weighing 12–19 (male) and 18–24 (female) g, were used instead of Swiss albino mice. Because of the low solubility of some compounds, 1 mg of each sample (I*a*–I*d*, II*a*–II*f*, and III*a*–III*e*) was given in equally divided doses subcutaneously 2 hr before and 4 hr after the injection of *S. aureus*[19]. 5-Aminovaleryl-L-histidine (V*d*) was

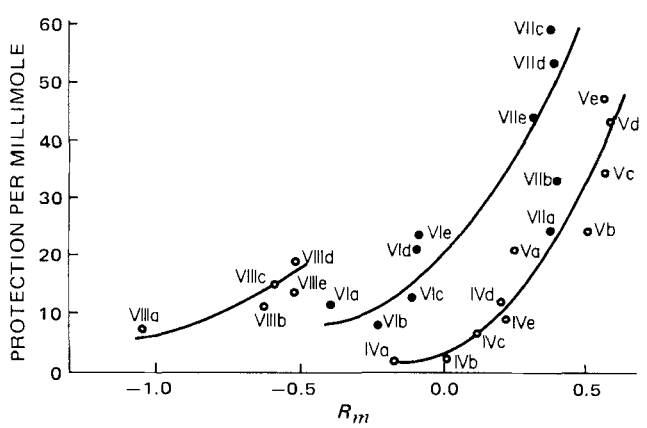

Figure 3—*Relationship between antistaphylococcal activity* in vivo *(protection per millimole) and* R_m *values of series IV–VIII.*

[13] Carbobenzoxy-β-alanine, Sigma Chemical Co.
[14] Carbobenzoxy-γ-aminobutyric acid, ICN Pharmaceuticals, Inc.
[15] Carbobenzoxy-δ-aminovaleric acid, prepared in this laboratory (2).
[16] Carbobenzoxy-ε-aminohexanoic acid, Sigma Chemical Co.
[17] Analtech, Inc.
[18] Polyethylene chamber (Analtech, Inc.).
[19] A clinical isolate, from a tonsilitis patient, carried in this laboratory since 1955 in the lyophilized state; see Ref. 35 for description.

Table I—R_f Values of Derivatives of Ia and IIa and of ω-Aminoacyl-4-aminobenzoic Acids on TLC [a] ($R_f \times 100$)

Compound	Solvent 1	Solvent 2	Solvent 3	Solvent 4	Solvent 5
Ia	66	87	86	—	92
Ib	45	62	79	33	61
Ic	41	65	76	63	91
Id	47	64	80	28	62
IIa	48	67	75	49	49
IIb	52	68	81	37	54
IIc	43	55	69	32	65
IId	52	67	81	32	55
IIe	37	59	71	31	68
IIf	55	66	80	34	60
IIIa	38	73	64	70	82
IIIb	38	69	61	40	79
IIIc	43	70	60	24	77
IIId	46	70	59	19	74
IIIe	49	72	61	14	77
L-Alanine [b]	35	40	52	47	71
L-Arginine [c]	13	22	36	4	25

[a] The plates were silica gel G (Merck), 250 μm, from Analtech, Inc. Solvent 1 = phenol–water (75:25 w/v), pH 2.0. Solvent 2 = 1-butanol–acetic acid–water (60:20:20), pH 2.4. Solvent 3 = 2-propanol–formic acid–water (77:4:19), pH 2.7. Solvent 4 = 1-methylpropanol–methyl ethyl ketone–dicyclohexylamine–water (55:15:10:20), pH 10.0. Solvent 5 = chloroform–methanol–17% NH_4OH (40:40:20), pH 11.4. [b] Spots were identified by ninhydrin reaction for Ia, Ic, IIa, IIc, IIe, and IIIa–IIIe, and L-alanine was the standard. [c] Spots were identified by Sakaguchi reaction for Ib, Id, IIb, IId, and IIf, and L-arginine was the standard.

used as the positive control. Compounds Ia, Ic, IIa, IIc, and IIe also were tested at 5 mg with a positive control of 4-aminobutyryl-L-histidine (Vc). Guanidino derivatives Ib, Id, IIb, IId, and IIf were poorly soluble in water. To provide a high dose of a compound of low water solubility, a fine suspension of IIf in cottonseed oil (20 mg/ml, dose of 0.25 ml) was also investigated.

Antistaphylococcal activity *in vivo* (ASA) is expressed as:

$$\text{ASA} = [(M_c - M_e)/M_c]/C \qquad \text{(Eq. 2)}$$

where M_c is the mortality of untreated negative control, M_e is the mortality of the experimental (treated), and C is the dose (millimoles). Previously (1–5), percent protection, $(M_c - M_e)100/M_c$, was used for comparison of activity. The relationship between antistaphylococcal activity and the R_m value of series I–III is summarized in Fig. 4. Antistaphylococcal activity *in vitro* was determined in the same manner as before (1).

RESULTS AND DISCUSSION

Compounds Ia–Id, IIa–IIe, and IIIa–IIIe were homogeneous on TLC with five different solvent systems (Table I). Single spots were given with the ninhydrin reaction, iodine treatment, and potassium dichromate–sulfuric acid. Compounds Ia, Ic, IIa, IIc, IIe, and IIIa–IIIe were positive to the phenol–calcium hypochlorite color reaction (2).

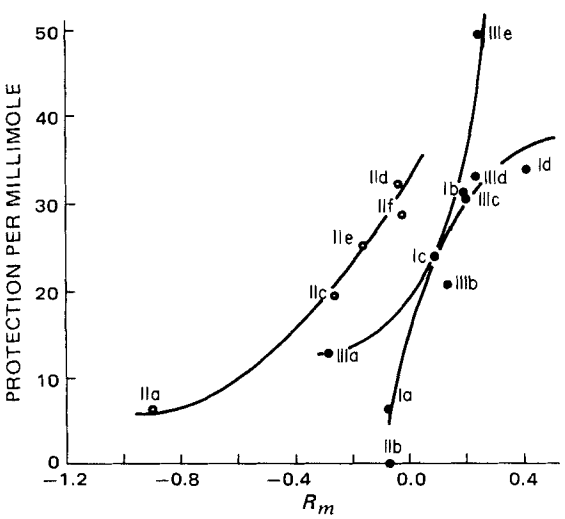

Figure 4—*Relationship between antistaphylococcal activity* in vivo *(protection per millimole) and* R_m *values of series I–III.*

Table II—Antistaphylococcal Activity *In Vivo* (ASA) and R_m Values of Probiotics

Compound [a]	ASA [b]	R_m [c]
Ia	6.44	−0.07 ± 0.11
Ib	31.53	0.19 ± 0.12
Ic	24.22	0.10 ± 0.08
Id	33.70	0.41 ± 0.14
IIa	6.17	−0.90 ± 0.10
IIb	0.00	−0.07 ± 0.12
IIc	19.53	−0.26 ± 0.11
IId	32.11	−0.04 ± 0.13
IIe	25.11	−0.16 ± 0.10
IIf	28.78	0.02 ± 0.14
IIIa	12.71	−0.29 ± 0.07
IIIb	20.72	0.14 ± 0.07
IIIc	29.66	0.21 ± 0.11
IIId	31.53	0.24 ± 0.08
IIIe	49.47	0.25 ± 0.09
IVa	1.80	−0.18 ± 0.07
IVb	2.32	0.01 ± 0.09
IVc	6.60	0.12 ± 0.09
IVd	13.12	0.20 ± 0.10
IVe	8.92	0.22 ± 0.10
Va	21.22	0.26 ± 0.11
Vb	23.98	0.51 ± 0.12
Vc	35.08	0.57 ± 0.13
Vd	43.57	0.59 ± 0.13
Ve	46.69	0.57 ± 0.08
VIa	11.71	−0.39 ± 0.10
VIb	8.39	−0.23 ± 0.06
VIc	12.77	−0.11 ± 0.07
VId	21.01	−0.09 ± 0.05
VIe	23.90	−0.08 ± 0.07
VIIa	24.50	0.38 ± 0.09
VIIb	33.21	0.40 ± 0.09
VIIc	59.34	0.38 ± 0.11
VIId	53.61	0.39 ± 0.10
VIIe	44.07	0.32 ± 0.10
VIIIa	7.11	−1.05 ± 0.10
VIIIb	11.26	−0.63 ± 0.11
VIIIc	15.31	−0.59 ± 0.10
VIIId	19.30	−0.52 ± 0.12
VIIIe	13.71	−0.52 ± 0.10

[a] L-Alanine was used as a positive control with an average R_m value of −0.30 ± 0.06 for ninhydrin-positive compounds Ia, Ic, IIa, IIc, IIe, and series III–VIII. L-Arginine was used as a positive control with an average R_m value of 1.32 ± 0.37 for Sakaguchi-reaction-positive compounds Ib, Id, IIb, IId, IIf, and series VI and VII. [b] ASA is defined by Eq. 2. [c] Silica gel G (Merck), 250-μm plates (Analtech, Inc.), were used. The stationary and mobile phases were 5% paraffin oil white in hexane (v/v) and 70% acetone in water (v/v), respectively.

The effectiveness of protection was calculated using the χ^2-test. All series I–III compounds, except Ia, IIa, IIb, and IIIa, were significantly effective as compared with the untreated negative control (>95% confidence level) at 1 mg. All of the compounds tested at 5 mg (Ia, Ic, IIa, IIc, and IIe) were significantly more effective than the untreated negative control (>95% confidence level). With a 5-mg dose as an oil suspension, IIf provided significant effectiveness at greater than the 99.5% confidence level. Antistaphylococcal activities *in vivo* obtained with two different dosages, 1 and 5 mg, were essentially identical (percent deviation <5.0), except for Ia which gave 31.1% deviation. None of the compounds had antistaphylococcal activity *in vitro*, as was true of the previously reported probiotics.

A regular relationship was obtained between the composition of the mobile phases and the R_m values (Figs. 1 and 2). The optimal mobile phase was determined to be 70% acetone in water, and it was used in the study of the correlation between structure and biological response. Antistaphylococcal activity *in vivo* and R_m values of series I–VIII compounds are summarized in Table II. In general, increased R_m values paralleled a corresponding increase in biological response to experimentally induced *S. aureus* infections in mice for each group of compounds investigated (Figs. 3 and 4). The histidine dipeptides of both ω-amino acids (series V) and ω-guanidino acids (series VII) showed higher R_m values and also higher antistaphylococcal activity than the corresponding parent acids.

The ω-amino acids have partition coefficients in 1-octanol–water of

the order of 10^{-3} [20]. The partition coefficient of IIa in the same system was reported as 4.8 (uncorrected) (36) or 2.9 (corrected) (37). It was hoped that the lipophilicity of the ω-amino acids would be enhanced by forming the ω-aminoacyl-4-aminobenzoic acids. The results on reversed-phase TLC indicate that no increase in lipophilicity of ω-amino acids resulted from forming IIa derivatives.

While there was a significant relationship between biological response and R_m values in each series of compounds, this was not true when all series I–VIII compounds were considered together. This finding suggests that the modifications of functions from amino (NH_2) to guanidino [$NCH(=NH)NH_2$], from carboxylic acid (COOH) to sulfonic acid (SO_3H), and from straight-chain polymethylene [$-(CH_2)_n-$] to ring (C_6H_6 or C_6H_{10}) gave a considerable change in partitioning. Therefore, each of the groups (series I–VIII) should be viewed independently.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 23, 1976, from the *Division of Chemistry and Biochemistry, St. Thomas Institute, Cincinnati, OH 45206, and the ‡Department of Chemistry, Xavier University, Cincinnati, OH 45207.

Accepted for publication August 13, 1976.

Presented in part at the 168th National Meeting of the American Chemical Society, Atlantic City, N.J., September 1974 (Abstract No. MEDI 69); the 169th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1975 (Abstract No. MEDI 42); and the 170th National Meeting of the American Chemical Society, Chicago, Ill., August 1975 (Abstract No. MEDI 59).

Abstracted in part from dissertations submitted by J. H. Bush and K. E. Shores to the Graduate School, Xavier University, in partial fulfillment of the Master of Science degree requirements.

Supported by a grant from Stanley Drug Products, Inc., Division of Sperti Drug Products, Inc.

The authors thank Dr. George S. Sperti, Dr. Leo G. Nutini, Dr. Kinji Tanaka, and Dr. Harvey A. Dube for their helpful suggestions. They also thank the Daiichi Seiyaku Co., Ltd., Tokyo, Japan, for a gift of *trans*-4-aminomethylcyclohexanecarboxylic acid.

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