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Amino Acid Analogs III: New Syntheses of Monomethyl- and Monophenylglutamic Acids

HERMAN GERSHON ˣ, RAULO PARMEGIANI, VINCENT R. GIANNASIO, and IRA S. KRULL

Abstract □ Glutamic acid analogs containing 3- and 4-methyl and 2-, 3-, and 4-phenyl substituents were prepared. The 3- and 4-methyl- and 3- and 4-phenylglutamic acids did not inhibit *Plasmodium berghei* and were nontoxic to the host (mice) at 640 mg/kg. The five analogs in addition to 2-methylglutamic acid were inactive against *Lactobacillus casei* at 1000 μg/ml in a defined medium; against *Escherichia coli*, only 2-methylglutamic acid caused 27% inhibition at 10,000 μg/ml. All six analogs failed to inhibit *Aspergillus niger, Aspergillus oryzae, Trichoderma viride*, and *Myrothecium verrucaria* in a defined medium below 10,000 μg/ml.

Keyphrases □ Glutamic acid analogs—syntheses, inhibition of plasmodia, bacteria, and fungi, toxicity in mice, potential antimalarial activity □ Antimalarial agents, potential—syntheses of methyl- and phenylglutamic acids

As a result of an interest in synthesizing potential antimalarial agents, it became necessary to prepare quantities of 3- and 4-methyl- and 3- and 4-phenylglutamic acids. The four glutamic acid analogs were reported previously (1–4).

DISCUSSION

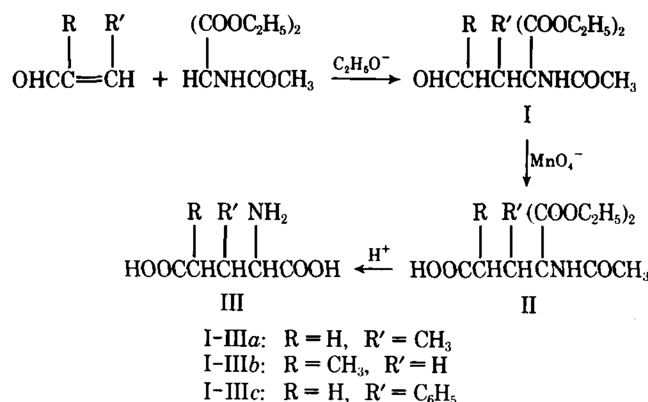
3-Methylglutamic acid (IIIa) was obtained by reacting crotonaldehyde with ethyl acetamidomalonate by means of a Michael condensation, followed by oxidation of the aldehyde function (I) with permanganate and hydrolysis of the oxidation product (II) with acid. The preparation of 4-methylglutamic acid (IIIb) was based on condensing methacrolein with ethyl acetamidomalonate, oxidizing, and hydrolyzing the products, as for 3-methylglutamic acid. 3-Phenylglutamic acid (IIIc) was obtained in a similar manner, by a Michael condensation of cinnamaldehyde with ethyl acetamidomalonate followed by oxidation and hydrolysis.

For the preparation of 4-phenylglutamic acid (V), tropic acid was made into the acid chloride by means of thionyl chloride which, in turn, was esterified with ethanol. Ethyl tropate was subsequently converted to ethyl 3-bromo-2-phenylpropionate with phosphorus tribromide, and the bromo compound was condensed with ethyl acetamidomalonate. Upon hydrolysis of the condensation product with acid, 4-phenylglutamic acid was obtained. Scheme I indicates the preparation of IIIa–IIIc, and Scheme II indicates the preparation of V.

Since it was desired to carry out microbiological and other testing on the three monomethyl- and three monophenylglutamic acids, 2-methyl- and 2-phenylglutamic acids were also required. The synthesis of 2-phenylglutamic acid was reported previously, but it cyclized spontaneously to 2-phenylpyroglutamic acid (5). For the present study, benzoylpropionic acid was treated with ammonium carbonate and potassium cyanide, according to the method of Henze and Speer (6), to form the hydantoin (VI). Compound VI was hydrolyzed to 2-phenylglutamic acid (VII) by successive treatments with sodium hydroxide and hydrochloric acid (Scheme III). This preparation of 2-phenylglutamic acid did not cyclize spontaneously at room temperature. 2-Methylglutamic acid was commercially available. IR spectra of the six glutamic acid analogs appear in Figs. 1 and 2.

3-Methyl-, 4-methyl-, 3-phenyl-, and 4-phenylglutamic acids were screened against *Plasmodium berghei* in mice[1]. These com-

[1] Testing done at the Rane Laboratory, University of Miami, Miami, Fla.



Scheme I

pounds at 640 mg/kg, the highest level administered, were both inactive to the *Plasmodium* and nontoxic to the host.

Since the bacterial cell wall of many species is known to contain D-glutamic acid (7–9), it was of interest to determine whether the substituted glutamic acids would inhibit bacterial growth, presumably by preventing the incorporation of D-glutamic acid into the bacterial wall. It was previously reported that when *Lactobacillus plantarum*, a glutamic acid-requiring mutant, was grown in a medium containing sufficient L-glutamic acid to afford half-maximal growth and the three monomethylglutamic acids were incorporated into the medium individually up to a molar ratio of 186:1, only 4-methylglutamic acid caused any inhibition of growth (10%) (10).

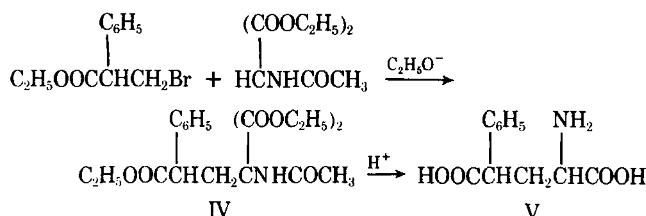
A test of the compounds against *Lactobacillus casei* (ATCC 9595), a second glutamic acid-requiring mutant, was carried out. Glutamic acid assay medium[2] enriched with 20 μg/ml of L-glutamic acid hydrochloride, allowed 85% of the optimal growth. No inhibition of the organism was observed when 1000 μg/ml of each of the six glutamic acid analogs was added to the assay medium. All six glutamic acid analogs were tested against *Escherichia coli* (ATCC 9723) in the medium of Davis and Mingioli (11). Only 2-methylglutamic acid inhibited the growth of the organism (27%) at a level of 10,000 μg/ml. All of these glutamic acid analogs also failed to inhibit four fungi, *Aspergillus niger* (ATCC 6275), *Aspergillus oryzae* (ATCC 1101), *Trichoderma viride* (ATCC 8678), and *Myrothecium verrucaria* (ATCC 9095), in Czapek–Dox agar[2] at pH 5.6 and 7.0 at 10,000 μg/ml, the highest level tested.

EXPERIMENTAL[3]

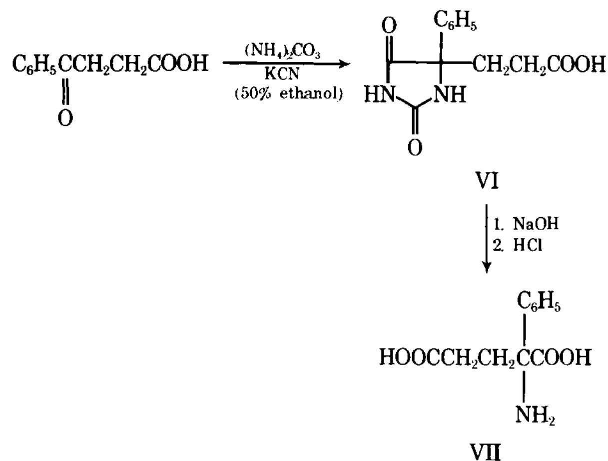
Ethyl 2-Acetamido-2-carbethoxy-3-methyl-5-oxovalerate (Ia)—This compound was prepared as reported previously (12), yielding 95%, mp 91–92°. An analytical sample was crystallized from ethanol, mp 95° [lit. (12) mp 88–89°].

Anal.—Calc. for $C_{13}H_{21}NO_6$: C, 54.35; H, 7.37; N, 4.88. Found: C, 54.15; H, 7.42; N, 4.90.

Ethyl 2-Acetamido-2-carbethoxy-3-methylglutarate (IIa)—Compound *Ia* (28.8 g, 0.1 mole) was dissolved in 380 ml of water at 70–75°. Potassium permanganate (22.5 g, 0.14 mole), dissolved in 450 ml of water, was added over 2 hr. The mixture was stirred for an additional 2 hr at 70–75°. The manganese dioxide was removed by filtration, and the filtrate was decolorized with a small quantity of sodium thiosulfate and refiltered. After acidification with dilute sulfuric acid, the solution was extracted with 5 × 100 ml of methylene chloride. The combined washings were backwashed with 50 ml of water and dried (sodium sulfate). The solid obtained upon evaporation of the solvent was crystallized from ether, yielding 15.5 g (56%), mp 100–103°. An analytical sample was crystallized from ether, mp 102–103°.



Scheme II



Scheme III

Anal.—Calc. for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.65; H, 7.12; N, 4.53.

DL-3-Methyl-DL-glutamic Acid (IIIa)—Compound II*a* (28.4 g, 0.1 mole) was heated under reflux with 285 ml of concentrated hydrochloric acid overnight. The excess acid was removed by flash evaporation, and the syrupy residue was diluted to 50 ml with water and adjusted to pH 3.2 with pyridine. Three volumes of ethanol was added to the solution, and the mixture was refrigerated at −10° overnight. The product was removed by filtration, washed with ethanol, and dried at 70° overnight, yielding 12.1 g (75%), mp 164° dec. [lit. (1) mp 164° dec.].

Ethyl 2-Acetamido-2-carbethoxy-4-methyl-5-oxovalerate (Ib)—Compound I*b* was prepared from methacrolein (technical grade contained 90% methacrolein and 10% acetic acid) and ethyl acetamidomalonate (12), and additional sodium was used to neutralize the acetic acid. The ethanol solution was evaporated to near dryness in a rotary evaporator, and the residue was dissolved in chloroform, washed free of salts (water), dried (sodium sulfate), and taken to dryness in the evaporator. The product was obtained as a syrup in 98% yield (12). An analytical sample was prepared by drying under vacuum overnight.

Anal.—Calc. for $C_{13}H_{21}NO_6$: C, 54.35; H, 7.37; N, 4.88. Found: C, 54.06; H, 7.31; N, 4.95.

Ethyl 2-Acetamido-2-carbethoxy-4-methylglutarate (IIb)—Compound II*b* was prepared from the aldehyde by oxidation with potassium permanganate in the same manner as II*a*. The yield was 43%, mp 121.5–122.5°. An analytical sample was crystallized from ether, mp 125.5–126°.

Anal.—Calc. for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.68; H, 6.75; N, 4.72.

DL-4-Methyl-DL-glutamic Acid (IIIb)—Compound III*b* was prepared by hydrolysis of II*b* with concentrated hydrochloric acid in the same manner as III*a*. The yield was 66%, mp 152° dec. An analytical sample was crystallized from aqueous acetone, mp 160.5° dec. [lit. (13) mp 154–155°].

Anal.—Calc. for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 45.06; H, 6.83; N, 8.56.

Ethyl 2-Acetamido-2-carbethoxy-5-oxo-3-phenylvalerate (Ic)—Compound I*c* was prepared from cinnamaldehyde and ethyl acetamidomalonate in the same manner as I*a*. The yield was 84%, mp 95–96°. An analytical sample was crystallized from ethanol, mp 96.5–97.5°.

Anal.—Calc. for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01. Found: C, 62.02; H, 6.62; N, 4.00.

Ethyl 2-Acetamido-2-carbethoxy-3-phenylglutarate (IIc)—Compound I*c* was oxidized to II*c* with potassium permanganate in the same manner as I*a*, except that the product crystallized from the filtrate upon acidification. It was obtained by filtration,

[2] Difco.

[3] Melting points were taken in a Thomas-Hoover melting-point apparatus and are uncorrected. IR spectra were obtained with a Perkin-Elmer model 221 spectrophotometer. 3-Benzoylpropionic acid, tropic acid, and 2-methylglutamic acid were obtained commercially

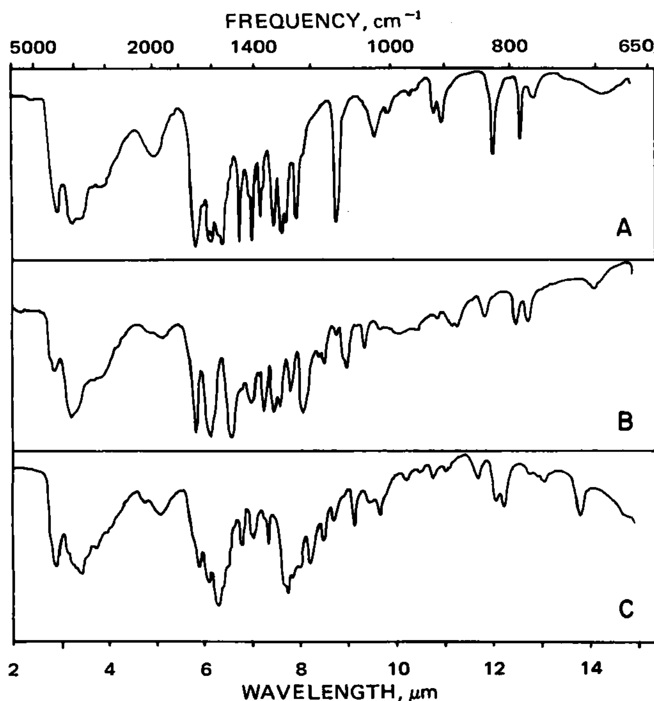


Figure 1—*IR spectra in KBr of* DL*-2-methylglutamic acid (A),* DL*-3-methyl-*DL*-glutamic acid (B), and* DL*-4-methyl-*DL*-glutamic acid (C).*

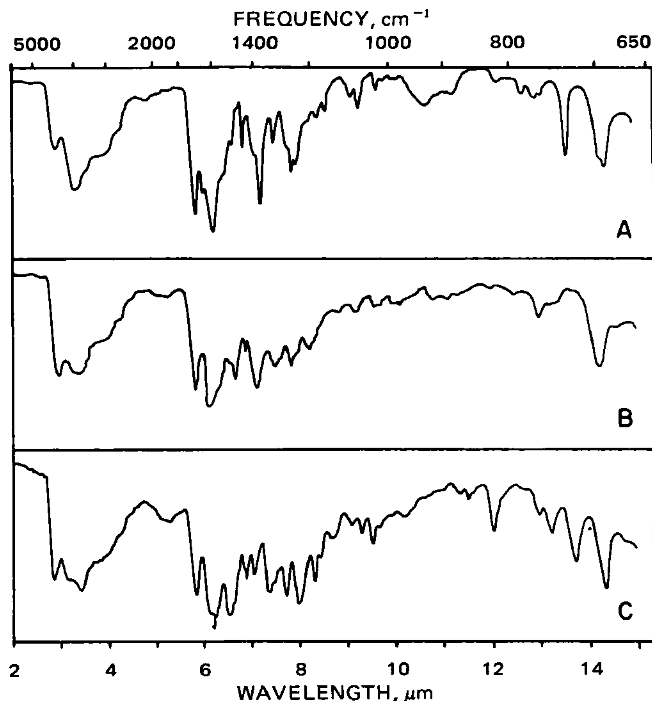


Figure 2—*IR spectra in KBr of* DL*-2-phenylglutamic acid (A),* DL*-3-phenyl-*DL*-glutamic acid (B), and* DL*-4-phenyl-*DL*-glutamic acid (C).*

washed free of salts with water, and dried at 70° overnight, mp 179–180° (56%). An analytical sample was crystallized from water, mp 180–180.5°.

Anal.—Calc. for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.34; N, 3.83. Found: C, 59.33; H, 6.29; N, 3.87.

DL-3-Phenyl-DL-glutamic Acid (IIIc)—Compound IIIc was prepared by hydrolysis of IIc with concentrated hydrochloric acid in the same manner as IIIa. The yield was 89%, mp 179–180° dec. An analytical sample was crystallized from aqueous acetone, mp 187–189° dec. [lit. (3) mp 175° dec.].

Anal.—Calc. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.59; H, 6.03; N, 6.01.

Ethyl Tropate—Tropic acid (500 g, 3.0 moles) was heated with thionyl chloride (1300 ml) under reflux with stirring and exclusion of moisture for 4 hr. The excess thionyl chloride was removed by flash evaporation, and the residue was heated with 2 liters of ethanol under reflux with stirring overnight. The alcohol was removed by flash evaporation, and the residue was distilled under vacuum. The yield of product was 524 g (90%), bp 120–124° (2 mm) [lit. (4) bp 152–154° (12 mm)].

Ethyl 3-Bromo-2-phenylpropionate—To ethyl tropate (194 g, 1.0 mole) in 200 ml of dry chloroform was added 100 g (0.36 mole) of phosphorus tribromide dropwise with stirring; the temperature was kept between −5 and 10°. The reaction mixture was stirred overnight and allowed to come to room temperature. It was again cooled to 0°, added slowly with stirring to a slurry of ice and water, extracted with chloroform, and washed twice with sodium bicarbonate followed by water. The chloroform layer was separated, dried (sodium sulfate), and freed of solvent by flash evaporation. The residue was distilled under vacuum, yielding 206 g (80%), bp 97–100° (0.5 mm) [lit. (4) bp 102° (0.5 mm)].

Ethyl 2-Acetamido-2-carbethoxy-4-phenylglutarate (IV)—To a solution of 4.5 g (0.196 g-atom) of sodium in 500 ml of anhydrous ethanol was added ethyl acetamidomalonate (42.5 g, 0.196 mole). A solution of ethyl 3-bromo-2-phenylpropionate (47.6 g, 0.196 mole) in 100 ml of dry ethanol was added dropwise with agitation. Upon completion of addition of the bromo compound, the mixture was kept under reflux with stirring overnight. The salts were removed by filtration, and the solvent was flash evaporated. The residue was dissolved in chloroform, washed twice (water), and dried (sodium sulfate). After evaporation of the solvent, the residue was dissolved in isopropanol and kept at −15° for several days. A yield of 67 g (85%) was obtained, mp 68–70°. An analytical sample was crystallized from isopropanol, mp 68.5–70° [lit. (4) mp 95°].

Anal.—Calc. for $C_{20}H_{27}NO_7$: C, 61.06; H, 6.92; N, 3.56. Found: C, 61.18; H, 6.91; N, 3.53.

DL-4-Phenyl-DL-glutamic Acid (V)—Compound IV (32.0 g, 0.081 mole) was heated under reflux in 320 ml of concentrated hydrochloric acid overnight, and excess acid was removed in a rotary evaporator. The residue was brought to pH 3.2 with pyridine and diluted with two volumes of ethanol. After refrigeration for several hours, the product was obtained by filtration, washed free of chloride with ethanol, and dried at 70° overnight. The yield of compound was 14.8 g (81%), mp 170–171° dec.

After recrystallization from aqueous acetone followed by vacuum drying at room temperature, the compound melted at 177–179° dec. Upon further heating, the material solidified and remelted at 203–204° (pyroacid). Additional recrystallizations yielded products of higher melting points. All possessed the same retention time by GC (trimethylsilyl derivative on 1% Apiezon column) [lit. (4) no melting point reported].

Anal.—Calc. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.35; H, 5.61; N, 6.20.

5-Phenyl-5-(2-carboxyethyl)hydantoin (VI)—A solution of 3-benzoylpropionic acid (100 g, 0.56 mole), ammonium carbonate (215 g, 2.24 moles), and potassium cyanide (72.8 g, 1.12 moles) in 1400 ml of 50% aqueous ethanol was kept at 60° for 2 hr. After the solution was cooled in an ice bath for several hours, the product crystallized and was removed by filtration, washed with a small volume of cold water, and dried at 100° under vacuum overnight. The yield was 131 g (94%), mp 214–215°. An analytical sample was crystallized from water, mp 215.5–216.5°.

Anal.—Calc. for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.28; O, 25.78. Found: C, 57.98; H, 5.05; N, 11.12; O, 25.98.

DL-2-Phenylglutamic Acid (VII)—A solution of 10 g (0.04 mole) of VI and 2.5 g (0.06 mole) of sodium hydroxide in 100 ml of water was kept at 160° in a stainless steel bomb for 16 hr. The bomb was cooled, and the solution was removed and boiled for 5 min to remove free ammonia, brought to pH 4 with acetic acid, and again boiled for 5 min to remove carbon dioxide. It was then passed through an ion-exchange resin[4] column (acidic) to remove sodium ions.

[4] Amberlite IR-120.

The column was washed with 1500 ml of hot water, the effluent was brought to near dryness by flash evaporation, and the residue was heated under reflux for 2.5 hr with 50 ml of concentrated hydrochloric acid. The hydrochloric acid was removed in a rotary evaporator, and the residual solution was dissolved in a small volume of water, decolorized with carbon, and adjusted to pH 3.1 with pyridine; then three volumes of ethanol was added.

After cooling at −15° overnight, the crystalline product was removed by filtration, washed with ethanol followed by ether, and dried at 70° overnight. The yield was 4.3 g (48%), mp 162–164°. On further heating, the melt solidified and remelted at 202–203° (2-phenylpyroglutamic acid). The analytical sample was crystallized from aqueous ethanol, mp 166–167° [lit. (5) mp 170°]; with further heating, the melting point was 205–206° [lit. (5) mp 207°].

Anal.—Calc. for $C_{11}H_{14}NO_4$: C, 59.19; H, 5.87; N, 6.27; O, 28.67. Found: C, 59.36; H, 5.95; N, 6.14; O, 28.89.

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Inhibitory Effect of Dioctyl Sodium Sulfosuccinate on Trypsin Activity

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Abstract □ The inhibitory effect of dioctyl sodium sulfosuccinate on the proteolytic activity of trypsin was investigated over the pH 6–8 range. The antitryptic activity was determined using two different substrates: casein and *N*,α-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride. The mechanistic studies revealed the substrate–inhibitor interaction to be the overall major mechanism of inhibition. This interaction was shown to involve substrate depletion, probably involving some primary sites of the natural substrate casein. Some inhibition was also shown to be due to an interaction between the enzyme and the inhibitor molecules. The interactions of the inhibitor with the enzyme and the substrate were irreversible. The possible therapeutic significance of the inhibitory effect of the surfactant is discussed.

Keyphrases □ Dioctyl sodium sulfosuccinate—trypsin inhibition, pH 6–8, two different substrates, therapeutic significance □ Trypsin—inhibition by dioctyl sodium sulfosuccinate, pH 6–8, two different substrates, therapeutic significance □ Enzyme inhibition—trypsin by dioctyl sodium sulfosuccinate, pH 6–8, two different substrates, therapeutic significance

The theories on the role of proteolytic enzymes in physiological and pathological states in humans and animals have been the topic of great controversy. The old belief that these enzymes catalyze protein synthesis no longer exists, yet it would be equally unjustified to restrict the role of body proteinases to a few processes such as cleavage of food proteins, mobilization of tissue proteins, and protein degradation usually associated with physiological wear and various traumas. In fact, it is now evident that these enzymes play a causative or adjunctive role in some disease processes such as inflammation, thromboembolic disorders, complement-dependent immune reactions, peptic and duodenal ulcers, and pancreatitis and even in some syndromes of malignant carcinomas (1).

Trypsin and kallikrein, which are present in the body tissues and fluids, are capable of releasing pharmacologically active plasma kinins (2). These proteolytic enzymes play an indirect but significant role in such important conditions as hypertension, shock, certain pains, changed capillary permeability, edema, and leucocyte migration. In the light of this knowledge, the proteinase inhibitors, both synthetic and natural, have gained new theoretical and practical importance (3). Recent attempts have been made to synthesize some active-site-oriented inhibitors as well as to improve their inhibitory activity (4–6). Also, it is reported that proteolytic enzymes are inhibited by agents such as sodium lauryl sulfate (7) and some sulfated polysaccharides (8).

Dioctyl sodium sulfosuccinate is an anionic surfactant, widely used medicinally as a fecal softener. The effect of this surfactant on drug absorption through membranes of varying complexities has been re-