New Amino Acids from *Streptomyces**

A. D. Argoudelis, R. R. Herr, D. J. Mason, T. R. Pyke, and J. F. Zieserl

ABSTRACT: Two new amino acids, 2-amino-3-dimethylaminopropionic acid and 2-amino-4,4-dichlorobutyric acid, have been isolated from fermentations of *Streptomyces* species. Both compounds, which have the L configuration, have been found to inhibit the growth of several bacteria grown in synthetic media. Isolation of these compounds and work supporting the proposed structures for these antimetabolites are discussed.

In the course of studies related to the production of antibiotics and other secondary metabolites from actinomycetes, we have isolated two new amino acids which have been found to inhibit growth of several microorganisms grown in synthetic media. 2-Amino-3-dimethylaminopropionic acid, the first of these new compounds, has been isolated from fermentations of *Streptomyces neocaliberis* var. *neocaliberis*[1] while the other, 2-amino-4,4-dichlorobutyric acid is produced by *Streptomyces armentosus* var. *armentosus*.[1] The present paper describes the production, isolation, chemical properties, and the structure of these compounds. Some of the biological properties are presented in this paper. However detailed studies of the behavior of both compounds will be presented in subsequent communications.

Experimental and Results Section

General. Infrared spectra were obtained in mineral oil suspension. Nuclear magnetic resonance (nmr) spectra were observed with a Varian A-60 spectrometer on solutions (*ca.* 0.4 ml, *ca.* 0.25 M) of the compounds in deuterium oxide. Spectra were calibrated in cycles per second at 60 Mcycles, downfield from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

Paper Chromatography. Paper chromatograms were run using descending development techniques. Solvent systems used were as follows: (I) 1-butanol–water (84:16), developed 16 hr; (II) 1-butanol–water (84:16) plus 0.25% *p*-toluenesulfonic acid, developed 16 hr; (III) 1-butanol–acetic acid–water (2:1:1), developed 16 hr; (IV) 1-butanol–water (84:16) plus 2% piperidine, developed 16 hr; (V) 1-butanol–water (4:96), developed 5 hr; and (VI) 1-butanol–water (4:96) plus 0.25% *p*-toluenesulfonic acid, developed 5 hr. 2-Amino-3-dimethylaminopropionic and 2-amino-4,4-dichlorobutyric acids were detected by biautography on *Pseudomonas mildenbergii* and *Proteus mirabilis* seeded agar, respectively. Both compounds were also located by using ninhydrin or periodate–permanganate sprays. The R_F values in the above-described solvent systems are as follows: 2-amino-3-dimethylaminopropionic acid (I) 0.07, (II) 0.10, (III) 0.45, (IV) 0.15, (V) 0.90, and (VI) 0.90; 2-amino-4,4-dichlorobutyric acid (I) 0.25, (II) 0.40, (III) 0.70, (IV) 0.40, (V) 0.85, and (VI) 0.82.

Thin Layer Chromatography. Thin layer plates were prepared from silica gel G (Merck Darmstadt). Thickness of the film was 0.4 mm. The solvent systems used were the following: (I), 70% aqueous ethanol; (II), methyl ethyl ketone–acetone–water (150:50:20, v/v); and (III), 95% ethanol–30% ammonia (77:33, v/v). Detection systems used were the same as those described above. The R_F values in the above described systems are as follows: 2-amino-3-dimethylaminopropionic acid (I) 0.07, (II) 0.00, and (III) 0.58; 2-amino-4,4-dichlorobutyric acid (I) 0.66, (II) 0.07, and (III) 0.68; homoserine (I) 0.50, (II) 0.00, and (III) 0.52; and DL-4-azaleucine (I) 0.07, (II) 0.00, and (III) 0.58.

2-Amino-3-dimethylaminopropionic Acid

Fermentation. Preseed flasks were inoculated with a spore preparation of the producing culture into 100 ml of a medium consisting of glucose monohydrate (Cerelose), 25 g/l. and Pharmamedia, 25 g/l. The culture was incubated for 72 hr at 28° on a rotary shaker. This preseed culture (100 ml) was used to inoculate a 30 l. of fermentor containing 20 l. of the same medium used for the preseed stage. After 25-hr incubation at 28°, 5% of the seed culture was transferred to 250 l. of medium in a 300-l. tank. The fermentation medium consisted of Pharmamedia, 26 g/l., corn starch, 25 g/l., $CaCO_3$, 5 g/l., and lard oil, 5 ml/l. The pH was adjusted to 7.2 prior to sterilization. The fermentation was continued for 44 hr at 28° with agitation at 260 rpm and air flow rate of 100 sec^{-1} $l.^{-1}$ $mole^{-1}$. 2-Amino-3-dimethylaminopropionic acid levels were determined with a disk-plate agar diffusion assay. Samples (0.08 ml) in pH 7.0, 0.1 M phosphate buffer were placed on 12.7-mm diameter paper disks and were assayed against *P. mildenbergii*. The results were expressed in biounits. A biounit is defined as the amount of activity necessary to produce a 20-mm diameter zone of inhibition against a sensitive microorganism inoculated into a

---

* From The Research Laboratories of the Upjohn Company, Kalamazoo, Michigan. *Received September 6, 1966.*

[1] Taxonomy of the organisms will be presented in a subsequent communication.

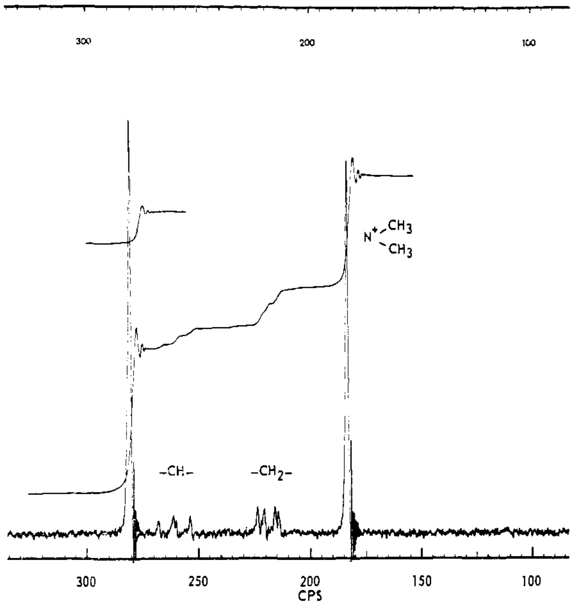


FIGURE 1: Nmr spectrum of L-2-amino-3-dimethylaminopropionic acid.

suitable agar medium and incubated for 20 hr at 28°. The following data were obtained: no yield at 0 hr and pH 7.4; 1.8 biounits at 20 hr and pH 6.9; and 10.4 biounits at 44 hr and pH 6.8.

Isolation. RECOVERY FROM FERMENTATION BROTH. The culture broth (239 kg), from a fermentation as described above, was filtered using filter aid. The filtered beer was adjusted to pH 3.0 with aqueous hydrochloric acid and allowed to stand at room temperature for 30 min. Insoluble material was removed by filtration, and the filtrate was adjusted to pH 7.0 with aqueous sodium hydroxide solution. The neutral filtered beer was passed through a column containing 10 l. of cation-exchange resin (Dowex 50) in the hydrogen form. The resin column was then washed with water and eluted with 10% aqueous ammonium hydroxide. Eluent fractions were analyzed for biological activity using the organism *P. mildenbergii*. Active fractions were pooled, concentrated *in vacuo* to remove ammonia, and freeze dried to give 186 g of crude preparation containing 2-amino-3-dimethylaminopropionic acid.

Isolation of Crystalline 2-Amino-3-dimethylaminopropionic Acid. CHROMATOGRAPHY. Material (357 g), isolated as described, was dissolved in 5.3 l. of water. The solution was adjusted to pH 4.0 with 5 N aqueous hydrochloric acid and filtered. The filtrate was adjusted to pH 7.0 with aqueous sodium hydroxide and then passed over a cation-exchange column containing 17 l. of Dowex 50 in the hydrogen form. The column was washed with water and eluted with 27 l. of each of the following solutions: 0.0625, 0.125, 0.25, 0.5, and 1.0 N aqueous ammonium hydroxide. The eluates were analyzed by paper chromatography and microbiological assay. Active fractions, concentrated to dryness, afforded 25 g of material containing other ninhydrin-positive compounds in addition to 2-amino-3-dimethylaminopropionic acid.

A portion (20.0 g) of the material isolated as described above was dissolved in 350 ml of water. This solution was stirred for 30 min with 70 g of activated carbon and the mixture was then filtered. The filtrate was freeze dried to afford 5.5 g of colorless material. An additional amount of 0.9 g of the same material was obtained by washing the carbon cake with water and freeze drying the aqueous solution. This material (6.4 g) was dissolved in 60 ml of water, adjusted to pH 6.0, and chromatographed over a column containing 400 ml of Dowex 50W in the hydrogen form. The column was eluted with aqueous ammonium hydroxide solution of increasing normality (from 0.0670 to 1 N). Fraction of 20 ml each were collected. The eluates were analyzed by paper chromatography and by spotting on *P. mildenbergii* seeded agar. Fractions 24–34 obtained by elution of the column with 0.25 N aqueous ammonium hydroxide were combined. This solution was mixed with equal volumes of 1-butanol and concentrated azeotropically to a volume of approximately 30 ml. Addition of absolute ethanol (175 ml) and acetone (175 ml) resulted in crystallization of 2-amino-3-dimethylaminopropionic acid which was isolated by filtration and dried (yield 2.5 g). An additional 0.5 g was obtained from the mother liquors, $[\alpha]_D^{25}$ -17.8 (*c* 1, water) and $+18.8°$ (*c* 1, aqueous HCl, pH 3.0). The infrared spectrum (in mineral oil suspension) shows absorptions at the following frequencies: 3400, 3340, 2950, 2920, 2850, 2810, 2760, 2720, 2610, 1600, 1508, 1460, 1430, 1412, 1404, 1375, 1340, 1325, 1288, 1268, 1165, 1133, 1098, 1075, 1045, 1032, 978, 876, 856, 812, 777, and 703 cm^{-1}.

Anal. Calcd for $C_5H_{12}N_2O_2$: C, 45.49; H, 9.16; N, 21.22; O, 24.24. Found: C, 44.68; H, 9.68; N, 20.08.[2]

2-Amino-3-dimethylaminopropionic Acid Sulfate. 2-Amino-3-dimethylaminopropionic acid (200 mg) was dissolved in a mixture of 3 ml of 1 N aqueous sulfuric acid, 3 ml of water, and 18 ml of methanol. Acetone was added until the solution became cloudy. The precipitated crystalline sulfate was isolated by filtration and dried, yield 65 mg. The nmr spectrum of 2-amino-3-dimethylaminopropionic acid sulfate is presented in Figure 1.

Anal. Calcd for $C_5H_{12}N_2O_2 \cdot H_2SO_4$: C, 26.11; H, 6.14; N, 12.18; S, 13.94. Found: C, 26.28; H, 6.13; N, 11.94; S, 13.77.

2-Amino-4,4-dichlorobutyric Acid

Fermentation. Fermentation conditions for 2-amino-4,4-dichlorobutyric acid were the same as those for 2-amino-3-dimethylaminopropionic acid with the exception of the fermentation medium which had the follow-

---

[2] We were unable to obtain good analytical values for 2-amino-3-dimethylaminopropionic acid. However, the analytical data for the sulfate salt are in excellent agreement with the calculated values.

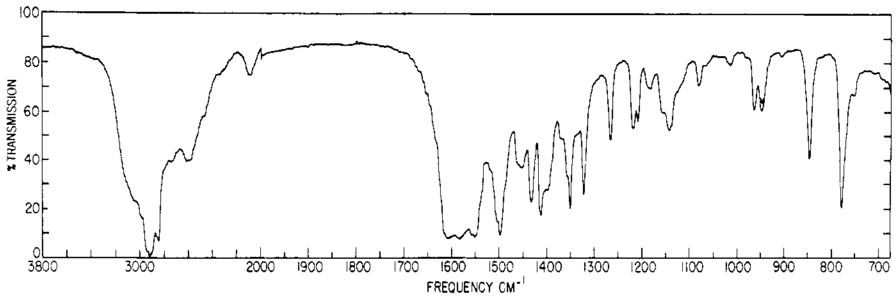


FIGURE 2: Infrared spectrum of L-2-amino-4,4-dichlorobutyric acid.

ing composition: starch, 30 g/l.; molasses, 20 g/l.; Pharmamedia, 15 g/l.; fish meal, 15 g/l.; and lard oil, 5 ml/l. The fermentation was incubated at 28° for 4 days with agitation at 280 rpm and air flow rate of 100 sec^{-1} l.^{-1} mole^{-1}. 2-Amino-4,4-dichlorobutyric acid was assayed by a disk-plate agar diffusion method similar to that described for the assay of 2-amino-3-dimethylaminopropionic acid. The assay organism was *P. mirabilis* growing in a synthetic medium. A typical fermentation had the following activity pattern: no yield at 0, 19, and 43 hr at pH 7.1, 6.5, and 6.7, respectively; 104 biounits at 67 hr and pH 6.9; 113 biounits at 91 hr and pH 6.9.

Recovery from the Fermentation Broth. The whole broth from the fermentation described above was filtered using filter aid. The filtered beer (286 l.) was concentrated *in vacuo* to a volume of 47 l. This concentrate was then stirred with 2.5 kg of activated carbon for 45 min. The mixture was filtered and the filtrate was mixed with 200 l. of acetone. The resultant precipitate was removed by filtration and discarded. The filtrate was concentrated to an aqueous solution which was freeze dried to give 1.9 kg of crude preparation containing 2-amino-4,4-dichlorobutyric acid.

Purification. EXTRACTION WITH METHANOL. The crude material (200 g), obtained as described, was mixed with 1200 ml of absolute methanol in a Waring blender for 10 min. The mixture was filtered and insoluble material was discarded. The filtrate (1 l.) was mixed with 5 l. of acetone. The precipitated material was separated by filtration and discarded. The remaining filtrate was concentrated to dryness to give 22.6 g of material which was purified further by countercurrent distribution as described below.

Countercurrent Distribution. The solvent system used consisted of equal amounts of 1-butanol and water. The starting material, 10 g of crude 2-amino-4,4-dichlorobutyric acid, was dissolved in 200 ml of the lower phase of the above system and this solution was introduced into 20 tubes of an all-glass Craig countercurrent distribution apparatus. Upper phase (200 ml) was added and the distribution was carried out for 1000 transfers. The distribution was analyzed by determination of the solids and by plating out dipped filter paper disks on agar medium seeded with *P. mirabilis*. Active fractions were combined, concentrated to an aqueous solution, and then freeze dried to give 480 mg of colorless material. An additional 870 mg was obtained by a similar distribution of 12 g of crude 2-amino-4,4-dichlorobutyric acid. The material obtained by countercurrent distribution (1.35 g) was dissolved with warming in 125 ml of absolute methanol and 7 ml of water. The clear solution was allowed to stand at 5° for 24 hr. Crystalline 2-amino-4,4-dichlorobutyric acid was separated by filtration and dried, yield 400 mg, $[\alpha]_D^{25}$ +6.7 (*c* 0.74, water) and +26.2° (*c* 0.74, aqueous HCl, pH 1.0). The crystalline material behaved as a single component in paper and thin layer chromatography. Countercurrent distribution showed a single peak with *k* value of 0.28. The infrared spectrum (in mineral oil suspension) (Figure 2) shows absorptions at the following frequencies: 3060, 2990, 2950, 2920, 2850, 2740, 2610, 2585, 2470, 2085, 1612, 1588, 1562, 1553, 1509, 1503, 1437, 1417, 1410, 1375, 1362, 1357, 1328, 1272, 1225, 1215, 1195, 1188, 1162, 1150, 1037, 1022, 971, 955, 914, 855, 785, 760, 675, and 642 cm^{-1}.

Anal. Calcd for $C_4H_7Cl_2NO_2$: C, 27.93; H, 4.10; Cl, 41.23; N, 8.14; O, 18.60; mol wt, 172. Found: C, 28.39; H, 4.44; Cl, 40.60; N, 9.09; O, 17.48 (by difference); mol wt (by potentiometric titration), 168 (titration with KOH) and 174 (in acetic acid solution, using perchloric acid as titrant).

Treatment of 2-Amino-4,4-dichlorobutyric Acid with Sodium Borohydride. A solution of 1.5 g of sodium borohydride in 25 ml of water was added to a solution of 500 mg of 2-amino-4,4-dichlorobutyric acid in 20 ml of 2.5 N aqueous sodium hydroxide. The mixture was heated on a steam bath. The reaction was followed by thin layer chromatography on silica gel G using 75% ethanol as the solvent system. Location of the spots was achieved by spraying with ninhydrin. After 3 hr the spot corresponding to 2-amino-4,4-dichlorobutyric acid had been replaced by another spot having an R_F value identical with that of homoserine. The mixture was then adjusted first to pH 2.0 with aqueous hydrochloric acid then to pH 7.0. This solution (70 ml) was mixed with 700 ml of absolute ethanol and 700 ml of ether. The precipitated material was discarded. The filtrate concentrated to dryness gave a colorless material which was purified by countercurrent distribution in the solvent system consisting of equal parts of 2-butanol and water. The distribution was analyzed

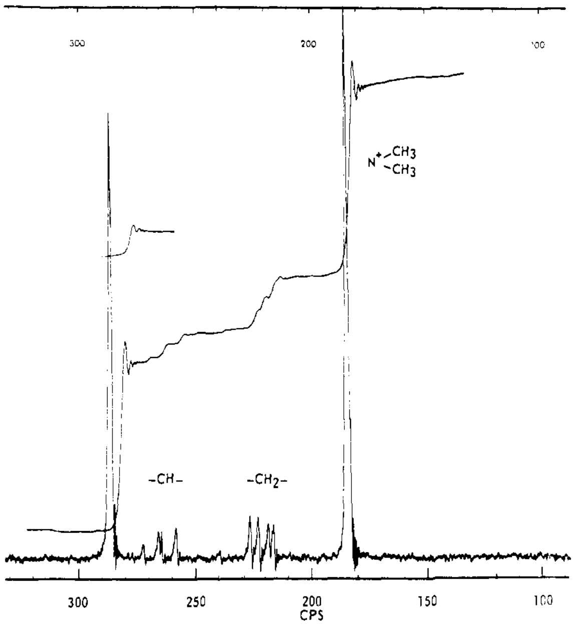
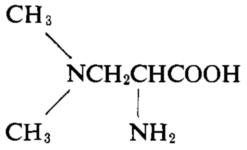


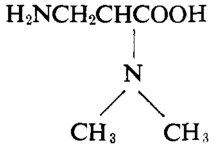
FIGURE 3: Nmr spectrum of DL-2-amino-3-dimethylaminopropionic acid (DL-4-azaleucine).

by thin layer chromatography. Combination of the tubes containing the homoserine-like material afforded 500 mg of colorless solid. Thin layer and paper chromatography of this material in several systems failed to separate it from homoserine. Quantitative determination using the Beckman-Spinco amino acid analyzer indicated the presence of 468 mcg of homoserine/mg of the colorless solid obtained.

Discussion

During studies related with the production of antibiotics and other secondary metabolites from microorganisms, it was observed that fermentation broth of *S. neocaliberis* var. *neocaliberis* contained a compound which exhibited antibacterial activity only against microorganisms grown in synthetic media. This compound (compound A) was isolated by ion-exchange chromatography as a colorless crystalline material having the molecular formula of $C_5H_{12}N_2O_2$. The infrared spectrum showed absorption bands at 2950 and 2610, 1600, 1508, and 1460 cm^{-1}, typical for a zwitterionic amino acid (Bellamy, 1960, p 234). The formation of a crystalline sulfate salt with the composition of $C_5H_{12}N_2O_2 \cdot H_2SO_4$ indicated the presence of two basic groups in compound A. Infrared spectrum of the sulfate showed carbonyl absorption band at 1762 cm^{-1} and several absorption bands at 2950–2660 cm^{-1}. The shift of the carbonyl absorption from 1600 to 1762 cm^{-1}, also suggests the amino acid structure for compound A. The nmr spectrum (Figure 1) of the sulfate salt showed absorptions at *ca.* 185, 222, and 263 cycles/sec due to six, two, and one protons, respectively. The sharp singlet at 185 cycles/sec (6 H) is assigned to a $(CH_3)_2N^+$ grouping. The complex absorptions centered at 222 cycles/sec (2 H) are due to a CH_2 group which is the AB part of an ABX system. This group must be attached to a charged nitrogen function to account for the shift of its absorption of frequencies higher than normally expected (Jackman, 1959, p 56). The absorption at *ca.* 263 cycles/sec (1 H) is assigned to the CH group which is the X part of the ABX system mentioned earlier. The frequency of this absorption indicates attachment of the methine group to an electronegative function (COOH). The data considered suggest structures I or II for compound A.


I


II

The DL racemate of 2-amino-3-dimethylaminopropionic acid (4-azaleucine, structure I) has been synthesized by Smith *et al.* (1963). Comparison of biological properties, paper and thin layer chromatographic patterns, and nmr spectra of compound A and those of the synthetic material (Figure 3) establish structure I as the structure of compound A. The large positive molecular rotation change (+48.3) observed upon passing from the neutral to the protonated form of compound A suggests the L configuration (Greenstein and Winitz, 1961), *i.e.*, compound A is L-2-amino-3-dimethylaminopropionic acid (L-4-azaleucine).

The second antimetabolite (compound B) to be discussed, produced in fermentations of *S. armentosus* var. *armentosus*, was isolated in crystalline form and found to have the molecular formula $C_4H_7Cl_2NO_2$. Potentiometric titration indicated the presence of a basic group, $pK_a' = 8.28$ (equivalent weight, 168 and 174; calculated molecular weight, 172). The infrared spectrum (Figure 2) shows absorptions at 2950–2585, 1612, and 1588 cm^{-1} indicating a zwitterionic amino acid structure. A strong absorption band at 785 cm^{-1} can be attributed to a $\mathrm{C}<^{\mathrm{Cl}}_{\mathrm{Cl}}$ grouping (Bellamy, 1960, p 328). Compound B gave a positive ninhydrin but negative silver nitrate test. However, the hydrolysate obtained by treatment of compound B with alkali gave white precipitate of silver chloride. The nmr spectrum of compound B (Figure 4) showed the presence of three exchangeable hydrogens with additional absorption centered at 167 cycles/sec due to two protons and at 238 and 373 cycles/sec each due to one proton. The absorption at 373 cycles/sec (1 H) is assigned to a $\mathrm{CH}<^{\mathrm{Cl}}_{\mathrm{Cl}}$ grouping. The presence of the two strongly electronegative chlorine atoms causes the shift of the methine-hydrogen absorption to frequency higher than normally expected (Jackman, 1959, p 59). The fact that this proton absorption appears as a triplet indicates coupling to two hydrogens on a neighboring carbon. The pair of doublets at 238

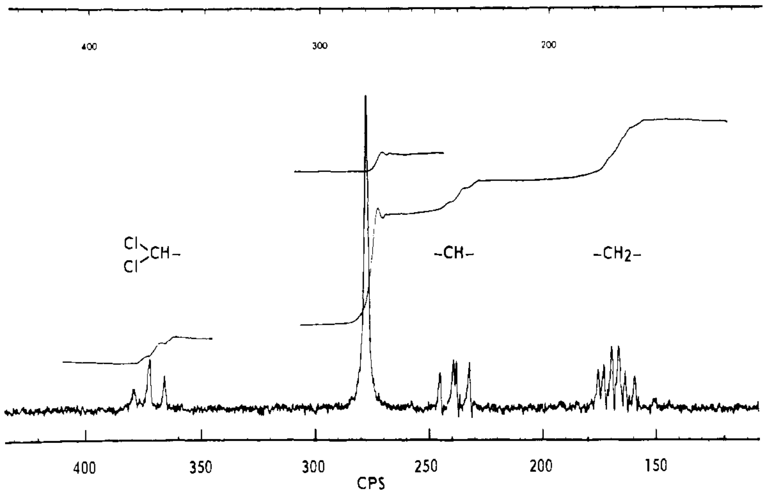
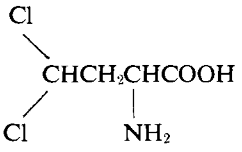


FIGURE 4: Nmr spectrum of L-2-amino-4,4-dichlorobutyric acid.

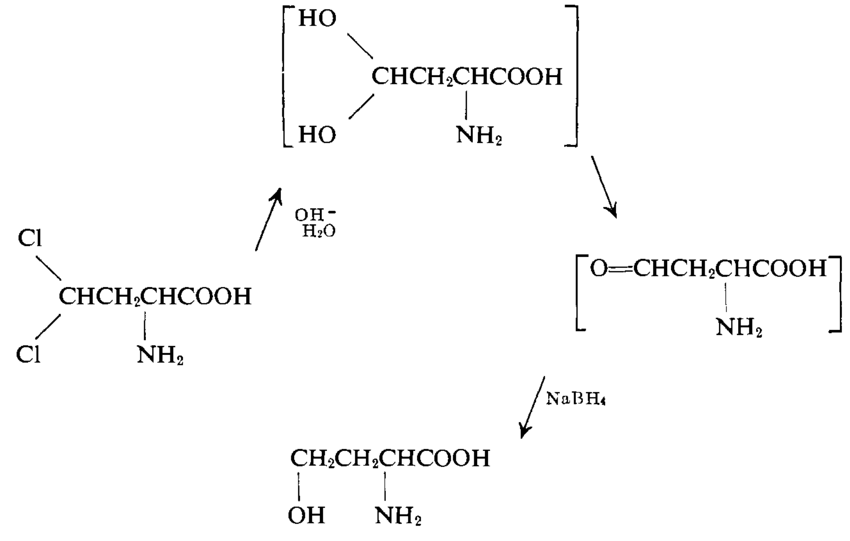
cycles/sec (1 H) is assigned to a methine group attached to both a COOH and a NH_2 group. This assignment accounts for the deshielding of the methine proton which, like the single proton discussed earlier is coupled to two neighboring hydrogens. Therefore, the complex absorptions centered at 167 cycles/sec are attributed to the two methylene hydrogens coupled to the single protons absorbing at 238 and 373 cycles/sec. Data presented so far suggest structure III for compound B.



$$\mathrm{Cl_2CHCH_2CH(NH_2)COOH}$$

III

The positive molecular rotation change (+33.6) observed by passing from the neutral to the protonated form of compound B indicates the L configuration and compound B is, therefore, L-2-amino-4,4-dichlorobutyric acid. Confirmation of this structure was obtained by treatment of compound B with alkali in the presence of sodium borohydride (Scheme I). The homoserine obtained was identified by thin layer and paper chromatography and determined quantitatively on a Beckman–Spinco amino acid analyzer. Both L-2-amino-3-dimethylaminopropionic acid and L-2-amino-4,4-dichlorobutyric acid were found to be moderately toxic when administered subcutaneously to mice with evidence of toxicity becoming apparent at levels of 80 and 25 mg/kg, respectively. 2-Amino-3-dimethylaminopropionic acid has been found active *in vitro* against *P. mildenbergii*, *Proteus rettgeri*, *Sarcina lutea*, and *Escherichia coli* while 2-amino-4,4-dichlorobutyric acid inhibited *E. coli*, *Proteus vulgaris*, and *P. rettgeri*. Preliminary studies indicate that both amino acids act as leucine antagonists. Detailed work on the mode



SCHEME I

of action of these antimetabolites will be reported in subsequent communications.

Acknowledgment

The authors wish to thank Mr. F. L. Cunningham and his associates for large-scale preparations and Dr. A. J. Parcells for his help in homoserine determinations.

References

Bellamy, L. J. (1960), The Infra-red Spectra of Complex Molecules, New York, N. Y., Wiley.

Greenstein, J. P., and Winitz, M. (1961), Chemistry of the Aminoacids, Vol. 1, New York, N. Y., Wiley, p 76.

Jackman, L. M. (1959), Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, New York, N. Y., Pergamon.

Smith, S. S., Bayliss, N. L., and MacCord, T. J. (1963), *Arch. Biochem. Biophys. 103*, 313.

Isolation, Characterization, and Synthesis of Linatine. A Vitamin B_6 Antagonist from Flaxseed (*Linum usitatissimum*)*

H. J. Klosterman, G. L. Lamoureux, and J. L. Parsons

ABSTRACT: The name linatine (I) was assigned to a vitamin B_6 antagonist isolated from linseed meal. Compound I inhibited the growth of chickens and *Azotobacter vinelandii* O, the assay organism. An LD_{50} of 2-mg per week-old chick was counteracted by simultaneous injection of 1 mg of pyridoxine. Acid hydrolysis of I yielded L-glutamic acid and a toxic substance (II), LD_{50} = 0.5 mg in 1-week-old chicks, which was characterized as 1-amino-D-proline, mp 155° dec, $[\alpha]^{25}_{\mathrm{D}}$ + 113° (*c* 2, 0.5 M HCl); 3-nitrobenzylidene derivative, mp 123°; 2-hydroxy-5-nitrobenzylidene derivative, mp 136°, $[\alpha]^{24}_{\mathrm{D}}$ +151° (*c* 0.35, ethanol). Compound II was prepared in 60% yield by reduction of 1-nitroso-D-proline with zinc dust in acetic acid. *N*-Carbobenzyloxyglutamic acid γ-hydrazide was converted to the azide and treated with the benzyl ester of II. Hydrogenolysis of the product yielded 1-[(*N*-γ-L-glutamyl)amino]-D-proline that was identical with I, $[\alpha]^{24}_{\mathrm{D}}$ +46.4° (*c* 2.75, water). 1-[(*N*-γ-Glutamyl)-amino]-L-proline prepared in the same manner showed $[\alpha]^{24}_{\mathrm{D}}$ −34.6° (*c* 2, water).

Both I and the diastereoisomer were equally toxic to chicks, but I was about 50 times more toxic toward *A. vinelandii* O.

Chicks and turkey poults show poor growth and typical vitamin B deficiencies when reared on linseed meal. Kratzer *et al.* (1954) noted that the vitamin deficiency symptoms could be prevented by the addition of 20 ppm of pyridoxine to linseed-containing diets and proposed that linseed meal contained a substance that was able to counteract the vitamin B_6 activity in an unsupplemented diet.

Klosterman *et al.* (1960) showed that the addition of a 70% ethanol extract of linseed meal to a standard ration produced poor growth and a vitamin B_6 deficiency. They also found that the substance responsible for poor growth could be absorbed from flaxseed extracts by cation-exchange resins and recovered by ammonia elution. Large-scale extraction of the toxic material from linseed meal has been described by Evenstad *et al.* (1965). This paper describes the isolation, characterization, and synthesis of the toxic vitamin B_6 antagonist from flaxseed. The trivial name linatine is suggested for this antagonist because of its association with the flax plant, *Linum usitatissimum*.

Experimental Section

Materials. N-Carbobenzoyloxyglutamic acid-γ-methyl ester was purchased from Mann Research Laboratories, Inc. D- and L-proline were obtained from Calbiochem. Dowex 1-X8 (Cl^- form), 200–400 mesh, was obtained from the Dow Chemical Co. and converted to the acetate form. Amberlite IRA-400 (Cl^- form) and Amberlite CG-120 (H^+ form), 100–200 mesh, were purchased from Mallinckrodt Chemical

---

* From the Departments of Agricultural Biochemistry and Bacteriology, North Dakota State University, Fargo, North Dakota. *Received August 22, 1966.* Presented in part at the Biochemical Section of the Great Lakes Regional Meeting of the American Chemical Society, Chicago, Ill., June 16–17, 1966. This research was supported in part by grants from the National Institutes of Health (AM 3024) and Abbott Laboratories. Published with the approval of the Director, North Dakota Agricultural Experiment Station, journal article no. 93.