Synthesis and Microbiological Activities of β-(1-Chloro-2-naphthyl)alanine and β-(1-Bromo-2-naphthyl)alanine

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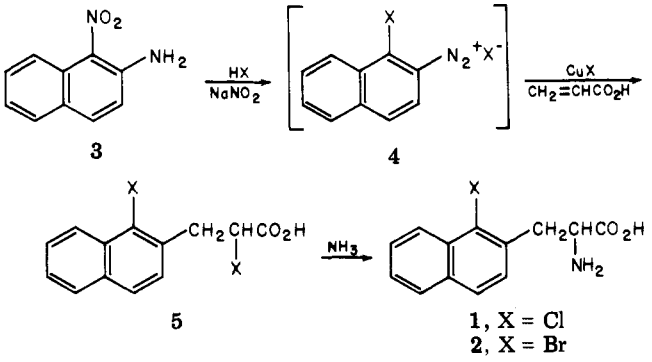
β-(1-Chloro-2-naphthyl)alanine and β-(1-bromo-2-naphthyl)alanine were synthesized by ammonolysis of the corresponding α,1-dihalo-2-naphthalenepropanoic acids derived from 1-nitro-2-naphthylamine by diazotization and condensation with acrylic acid in the presence of cuprous halides. The two analogs as well as the previously reported β-(2-naphthyl)alanine and β-(1-naphthyl)alanine were studied as growth inhibitors of *Escherichia coli* 9723, *Leuconostoc dextranicum* 8086, and *Lactobacillus plantarum* 8014. In general, the chloro and bromo analogs were more effective than the unsubstituted naphthylalanines as growth inhibitors of the three microorganisms studied.

In continuing our structure–activity studies of halo-substituted aromatic amino acids,[1] β-(1-chloro-2-naphthyl)alanine (1, X = Cl) and β-(1-bromo-2-naphthyl)alanine (2, X = Br)[2] have been prepared and examined for their activities in *Escherichia coli* 9723, *Leuconostoc dextranicum* 8086, and *Lactobacillus plantarum* 8014. Also, the activities of these analogs were compared to those of the unsubstituted isomeric compounds,[3] β-(2-naphthyl)alanine and β-(1-naphthyl)alanine, and to that of 2-chlorotyrosine[1] which was used as the assay standard.

The Meerwein arylation method of aromatic amino acid synthesis[4,5] was used to prepare the two halo analogs 1 and 2. The diazotization of 1-nitro-2-naphthylamine (3) under



3 4 5

1, X = Cl
2, X = Br

usual conditions resulted in the displacement of the nitro group by the halide ions to form the 1-halo-2-naphthalenediazonium halides (4), which in turn were condensed with acrylic acid in the presence of the appropriate cuprous halide to yield the corresponding α,-1-dihalo-2-naphthalenepropanoic acids (5). The halo acids 5 were subjected to ammonolysis in concentrated aqueous ammonia to give the respective β-(1-halo-2-naphthyl)-alanines 1 and 2.

The comparative activities of the four naphthylalanines and 2-chlorotyrosine in *E. coli*, *L. dextranicum*, and *L. plantarum* are given in Table I. For the two lactobacilli, the assay media contained minimal amounts of phenylalanine, tyrosine, and tryptophan as described in the Experimental Section. The testing of the naphthylalanines at levels higher than 200 μg/ml was precluded by their relatively low solubilities in the buffered assay media. The activities of the naphthylalanines were found to vary considerably in the different microorganisms. Under these assay conditions, the naphthylalanines were only moderately effective as growth inhibitors as compared to 2-chlorotyrosine.

Of the four naphthylalanines studied in *E. coli* at the limits of their solubilities, only the 1-bromo analog was active as a growth inhibitor. However, in *L. dextranicum* and *L. plantarum*, the 1-chloro analog was the most effective growth inhibitor. In particular, the 1-chloro analog inhibited the growth of both *L. dextranicum* and *L. plantarum* ten times more effectively than the 1-bromo analog and the unsubstituted β-(1-naphthyl)alanine. The β-(2-naphthyl)alanine was inactive in all three assay organisms even at concentrations of 200 μg/ml.

Table I. Comparative Microbiological Activities of Some Naphthylalanines and 2-Chlorotyrosine

Compound	MIC, μg/ml[a] *E. coli*[b]	*L. dextranicum*[b]	*L. plantarum*[b]
β-(1-Chloro-2-naphthyl)-alanine (1)	>200	20	20
β-(1-Bromo-2-naphthyl)-alanine (2)	200	200	200
β-(2-Naphthyl)alanine	>200	>200	>200
β-(1-Naphthyl)alanine	>200	200	200
2-Chlorotyrosine	2	6	0.6

[a] Minimum inhibitory concentrations. [b] Growth media and assay conditions as described in the Experimental Section.

The effects of a supplement of 40 μg/ml each of phenylalanine, tyrosine, and tryptophan upon the amounts of amino acid analogs necessary for inhibition of growth of *L. dextranicum* and *L. plantarum* were determined. For both microorganisms, the minimum inhibitory concentrations of β-(1-chloro-2-naphthyl)alanine, β-(1-bromo-2-naphthyl)alanine, and β-(1-naphthyl)alanine were not affected by the amino acid supplement. However, in the presence of the supplement, a 30-fold increase in the concentration (from 6 to 200 μg/ml) of 2-chlorotyrosine was required to inhibit the growth of *L. dextranicum* and a 300-fold increase in analog concentration (from 0.6 to 200 μg/ml) was ineffective in preventing the growth of *L. plantarum*. In contrast to the specific and competitive reversal of 2-chlorotyrosine inhibitions by tyrosine[1] in the amino acid supplement, the results obtained with the β-naphthylalanines indicate that they do not exert their inhibitory effects on the growth of *L. dextranicum* and *L. plantarum* by interfering with the metabolic functions of phenylalanine, tyrosine, or tryptophan as might be expected from their structural similarities.

The introduction of chloro and bromo substituents on the 1 position of β-(2-naphthyl)alanine has produced analogs which exhibit antibacterial activities that are not possessed by the parent amino acid itself. This study appears to be the first report of the antibacterial activities of naphthylalanines and suggests that other bioactive compounds may be found among this class of compounds.

Experimental Section

General. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Garden City, Mich. Where

analyses are indicated only by symbols of the elements, analytical results obtained by those elements were within ±0.4% of the theoretical values.

DL-α,1-**Dichloro-2-naphthalenepropanoic Acid.** To a mixture containing 9.41 g (0.05 mol) of 1-nitro-2-naphthylamine in 150 ml of acetone and 50 ml of concentrated HCl was added slowly a solution of 3.45 g (0.05 mol) of $NaNO_2$ in 20 ml of H_2O with stirring at 0°. Glacial acrylic acid (50 ml) was then introduced to the reaction mixture followed by careful addition of 600 mg of CuCl in small increments so as not to exceed a reaction temperature of 5°. When the N_2 evolution had ceased, the mixture was reduced in vacuo and then treated with 200 ml of aqueous 5% $KHCO_3$ to effect solution. The aqueous solution was then extracted with 100 ml of Et_2O and 100 ml of $CHCl_3$. The aqueous layer was separated, treated with activated charcoal, and filtered. The filtrate was acidified by the addition of concentrated HCl and the precipitate which formed was collected on a filter to give 9.21 g (68%) of crude product. Recrystallization from formic acid gave an analytical sample, mp 157–158°. Anal. ($C_{13}H_{10}Cl_2O_2$) C, H, Cl.

DL-α,1-**Dibromo-2-naphthalenepropanoic Acid.** Following the same procedure as that described above, 9.41 g (0.05 mol) of 1-nitro-2-naphthylamine in 150 ml of acetone and 60 ml of concentrated HBr were treated with 50 ml of glacial acrylic and 1.5 g of CuBr to yield 5.73 g (32%) of crude product, mp 171–178°. Recrystallization from HOAc–H_2O gave an analytical sample, mp 177–178°. Anal. ($C_{13}H_{10}Br_2O_2$) C, H, Br.

β-(1-**Chloro-2-naphthyl**)-DL-**alanine** (1). A 1.25-g (0.00464 mol) sample of DL-α,1-dichloro-2-naphthalenepropanoic acid was treated with 40 ml of concentrated NH_4OH at 70° for 60 h. The reaction mixture was evaporated to dryness in vacuo, and the resulting residue was washed with H_2O, acetone, ether, and hot benzene to yield 0.72 g (62%) of crude product, mp 249–251°. Recrystallization from acetic acid–ether gave an analytical sample, mp 256–257° dec. Anal. ($C_{13}H_{12}ClNO_2$) C, H, Cl.

β-(1-**Bromo-2-naphthyl**)-DL-**alanine (2).** A 1.00-g (0.00279 mol) sample of DL-α,1-dibromo-2-naphthalenepropanoic acid was treated with 100 ml of concentrated NH_4OH for 48 h at 0° and then for 48 h at 25°. The reaction mixture was evaporated to dryness in vacuo and the residual material was washed with H_2O, filtered, and air-dried to give 0.60 g (73%) of crude solid, mp 235–237° dec. The solid was washed with boiling acetone to obtain the desired product, mp 248–249° dec. Anal. ($C_{13}H_{12}BrNO_2$) C, H, N.

Microbiological Assays. The assay procedure[6] and inorganic salts–glucose medium[7] for *E. coli* (ATCC 9723) were the same as previously reported. For the lactic acid bacteria a previously described procedure and basal medium[8] were used except that 0.2 μg/ml of calcium pantothenate and 0.02 μg/ml of pantethine were included in the vitamin supplement, phenylalanine and tyrosine were omitted from the amino acid medium, and the concentration of tryptophan was decreased to 3 μg/ml. Additional modifications are noted for each organism. For *L. dextranicum* (ATCC 8086), the phosphate concentration was increased fourfold and the medium further supplemented with 100 μg/ml of glutamine. For *L. plantarum* (ATCC 8014), the concentrations of phenylalanine and tyrosine were reduced to 1 μg/ml.

The naphthylalanines (10 mg) were dissolved in sterile H_2O (10 ml) with slight warming. From these solutions, serial dilutions were made to the desired concentration and added aseptically to the previously autoclaved tubes. After inoculation, the assay tubes with *E. coli* were incubated at 37° for 16 h, and those with *L. dextranicum* and *L. plantarum* were incubated at 30° for 18 h.

In all assays the amount of growth was determined spectrophotometrically at 625 nm with a Bausch & Lomb Spectronic 20 in terms of absorbance readings of the turbid culture medium against a blank of uninoculated medium set at zero absorbance. Appropriate controls were run in all assays and the results of the minimum inhibitory concentrations of the various compounds and the standard (2-chlorotyrosine) were shown to be reproducible on repeating the assays at least six times.

Acknowledgment. The support of this work by research grants (R-285 and R-286) from the Robert A. Welch Foundation, Houston, Texas, is gratefully acknowledged. The authors are indebted to Dr. Floyd W. Dunn (Abilene Christian College) for supplying samples of β-(1-naphthyl)alanine and β-(2-naphthyl)alanine.

References and Notes

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Optical Resolution of (±)-2,5-Dimethyl-2'-hydroxy-9α- and -9β-propyl-6,7-benzomorphans and Their Pharmacological Properties

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The levo and dextro isomers of 2,5-dimethyl-2'-hydroxy-9α- and -9β-propyl-6,7-benzomorphans have been prepared. The analgesic potency and physical dependence capacity of the optical isomers and their racemic parents were determined. The 9α-propyl levo isomer was analgesically equipotent with morphine; the 9β-propyl levo isomer was considerably more potent subcutaneously and equipotent orally. None of the optical isomers suppressed the withdrawal syndrome; the 9β-propyl levo isomer exacerbated the withdrawal syndrome.

The separation of desired analgesia from physical dependence, one of the more serious side effects of morphine-like drugs, has been achieved with the benzomorphan molecule in at least two ways. (1) Substitution of certain saturated and unsaturated side chains for the methyl group on the nitrogen atom of this molecule has produced agonist–antagonists, as observed in both monkey species and in man.[1] (2) More recently, it has been shown that optical resolution produces one isomer (levo) that is without physical dependence capacity in rhesus monkeys and which may actually induce or exacerbate the abstinence syndrome.[2-4]

Only very occasionally have we observed antagonistic effects to narcotic analgesics in racemic *N*-methylbenzomorphans.[5-7] (These *N*-methyl racemic compounds are generally found in the 9α-alkylbenzomorphan series.)