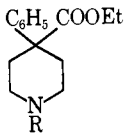
bamate ion intermediate.[5] The carbamate ion is unstable and is rapidly decarboxylated to free amine and CO_2. Normeperidine ethyl carbamate (III) was prepared for evaluation in the expectation that hydrolysis of the ethyl ester would be followed *in vivo* by a facile decarboxylation to normeperidine.

Normeperidine monosuccinamide (IV) was prepared for evaluation on the basis of the well-established facilitation of hydrolysis of monoamides of dicarboxylic acids capable of forming cyclic anhydrides.[6,7]

Normeperidine pyruvamide (V) was prepared in the expectation that pyruvamides, like pyruvate esters, would undergo facile hydrolysis. Electron-withdrawing substituents in the acid moiety of esters are known to accelerate the second-order alkaline hydrolysis rates, and the rate of alkaline hydrolysis of ethyl pyruvate was found to be particularly high.[8,9] Furthermore, Sudborough found ethyl pyruvate to undergo substantial hydrolysis even in water.[10]



I, R = CH_3
II, R = H
III, R = COOEt
IV, R = $COCH_2CH_2COOH$
V, R = $COCOCH_3$

Pharmacological Evaluation.—In the mouse hot plate test for analgesic activity, normeperidine ethyl carbamate (III) showed a mouse ED_{50} of 20.15 mg/kg (18.56–21.75) with a duration of 162 min.[11] The route of administration was subcutaneous and the figures in parentheses are the limits of error in probit analysis. In the hot wire tail-withdrawal test in the Wistar rat, III showed a rat ED_{50} of 64.0 mg/kg (33.7–121.6). The route of administration was oral. Meperidine was run side by side on a blind basis, and the two compounds were found to be approximately equipotent by this procedure.[12] The compound showed no toxicity or physical dependence capacity (capacity to suppress abstinence symptoms in morphine-dependent monkeys) at doses ranging from 1.0 to 130.0 mg/kg.[13] The interest in normeperidine ethyl carbamate (III) lies in its reasonably high potency order with no physical dependence capacity, in the range of doses tested.

Normeperidine monosuccinamide (IV) and normeperidine pyruvamide (V) showed no analgesic effect up to 100 mg/kg in the mouse hotplate test.[11] No physical dependence capacity or toxic effects were noted for either compound at doses of 2.0–40.0 mg/kg.[14]

(5) L. W. Dittert and T. Higuchi, *J. Pharm. Sci.*, **52**, 852 (1963).

(6) M. L. Bender, *J. Am. Chem. Soc.*, **79**, 1258 (1957); M. L. Bender, Y. Chow, and F. Chloupek, *ibid.*, **80**, 5380 (1958).

(7) T. Higuchi and T. Miki, *ibid.*, **83**, 3899 (1961); T. Higuchi, T. Miki, A. C. Shah, and A. K. Herd, *ibid.*, **85**, 3655 (1963).

(8) C. K. Ingold, "Structure and Mechanisms in Organic Chemistry," Bell and Sons, London, 1953.

(9) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940.

(10) J. J. Sudborough, *J. Chem. Soc.*, **101**, 1227 (1912).

(11) We are indebted to Dr. N. B. Eddy, National Institutes of Health, for these data; *cf.* N. B. Eddy and D. Leimbach, *J. Pharmacol. Exptl. Therap.*, **107**, 385 (1953).

(12) We are indebted to Dr. Maxwell Gordon, Smith Kline and French Laboratories, through whose courtesy these tests were carried out.

(13) We are indebted to Drs. G. A. Deneau and M. H. Seevers, University of Michigan, for these data.

Experimental Section[15]

Normeperidine Ethyl Carbamate (III).—To a stirred, ice-cooled solution of normeperidine hydrochloride (8.07 g, 0.03 mole, Winthrop, mp 134–137°) in $CHCl_3$ (25 ml) were added triethylamine (6.00 g, 0.06 mole) in $CHCl_3$ (15 ml) and (dropwise) ethyl chloroformate (3.24 g, 0.03 mole, Eastman) in $CHCl_3$ (15 ml). The solution was stirred in the cold for 2 hr and then at room temperature overnight. Ether was added to complete precipitation of triethylamine hydrochloride, and the precipitate was filtered. The filtrate was washed twice with water, dried (Na_2SO_4), and evaporated under reduced pressure to a light brown oily residue. The residue was dissolved in Skellysolve A and cooled and rubbed to induce crystallization. The crystalline product was recrystallized four times from Skellysolve A to yield 4.03 g of colorless crystals: mp 37–38°; $\lambda_{max}^{CHCl_3}$ 5.82 (ester C=O), 5.95 (carbamate C=O), 6.25, 14.40 (aryl ring), 8.0 μ (ester C–O–C).

Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.96; H, 7.63; N, 4.79.

Normeperidine Monosuccinamide (IV).—A solution of normeperidine hydrochloride (15 g, 0.055 mole) in water (25 ml) was treated with excess concentrated NH_4OH. The suspension was extracted three times ($CHCl_3$) and the extract was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was treated with succinic anhydride (5.5 g, 0.055 mole) and the mixture was heated on the steam bath for 1 hr. The oily reaction product was rubbed to induce crystallization, and then recrystallized twice from benzene: yield 12.80 g; mp 130–133°; $\lambda_{max}^{CHCl_3}$ 2.85 (OH), 5.82 (broad, ester and acid C=O), 6.10 (amide C=O), 6.25, 14.40 (aryl ring), 8.05 μ (ester C–O–C).

Anal. Calcd for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20. Found: C, 65.07; H, 7.09; N, 4.44.

Normeperidine Pyruvamide (V).—To a solution of pyruvic acid (3.52 g, 0.04 mole, Eastman, redistilled) in $CHCl_3$ (20 ml) was added $SOCl_2$ (4.76 g, 0.04 mole), and the solution was heated under reflux for 1 hr. To the stirred, refluxing solution, a solution of normeperidine hydrochloride (5.39 g, 0.02 mole) and triethylamine (2.02 g, 0.02 mole) in $CHCl_3$ (25 ml) was added dropwise over the course of 45 min, and refluxing was continued for an additional 75 min. The mixture was extracted three times with water, and the $CHCl_3$ layer was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. Crystallization from Skellysolve B yielded 4.68 g of product, mp 92–94°. Recrystallization from Skellysolve B, with Norit treatment, yielded the analytical sample: mp 94–96°; $\lambda_{max}^{CHCl_3}$ 5.82 (ester and ketone C=O), 6.10 (amide C=O), 6.25, 14.40 (aryl ring), 8.1 μ (ester C–O–C).

Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.22; H, 7.04; N, 4.79.

(14) G. A. Deneau and M. H. Seevers, Addendum to the Minutes of the Committee on Drug Addiction and Narcotics, National Academy of Sciences–National Research Council, 1965.

(15) Melting points, determined on a Fisher-Johns hot stage, are corrected. Infrared absorption spectra were determined in $CHCl_3$ on a Beckman model IR5A recording spectrophotometer. Microanalyses were carried out by Mr. J. F. Alicino, Metuchen, N. J. Skellysolve A refers to petroleum ether, bp 40–60°.

DL-4,5-Dihydroxy-2-pyridylalanine, an Analog of 3,4-Dihydroxyphenylalanine[1]

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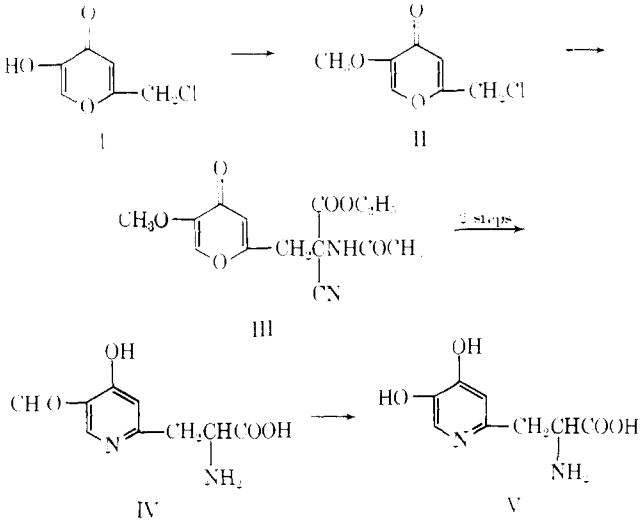
We wish to report the synthesis of DL-4,5-dihydroxy-2-pyridylalanine, a structural analog of 3,4-dihydroxyphenylalanine (DOPA). In previous studies amino acid analogs containing the pyridine ring in place of

(1) This work was supported in part by grants from The Robert A. Welch Foundation of Texas (Grant No. B-133) and from the U. S. Public Health Service (Grant No. AM 07599-04).

the benzene ring (2-pyridylalanine and 5-hydroxy-2-pyridylalanine, structural analogs of phenylalanine and tyrosine, respectively) have proved to be effective antimetabolites.[2,3] The substitution of the pyridine ring for the benzene ring in DOPA should, in all probability, provide an analog that would serve as an effective DOPA antagonist. The hydroxyl group on the 4 position of the pyridine ring of the analog probably exists both in the enol and keto forms; however, it would be anticipated that the analog in either the enol or keto form would serve as an antagonist of DOPA.

The synthesis of 4,5-dihydroxy-2-pyridylalanine was carried out using 2-chloromethyl-5-hydroxy-4H-pyran-4-one (chlorokojic acid, I) as starting material, and the sequence of reactions is shown in Scheme I. The con-

SCHEME I



densation product (III) from the reaction between sodioacetamidocyanoacetic ester and 2-chloromethyl-5-methoxy-4H-pyran-4-one (II) was treated with NH_4OH under heat and pressure to effect substitution of the heterocyclic oxygen atom with the heterocyclic nitrogen atom. The product, a dark viscous oil, gave a strong ninhydrin reaction (indicating at least partial hydrolysis); however, all efforts to obtain a crystalline material were unsuccessful. Further hydrolysis in alkaline solution yielded DL-4-hydroxy-5-methoxy-2-pyridylalanine (IV). The desired product, DL-4,5-dihydroxy-2-pyridylalanine (V), was obtained by cleavage of the 5-methoxy group with concentrated HI.

Biological Data. The effect of DL-4,5-dihydroxy-2-pyridyl alanine on the oxidation of DL-DOPA by tyrosinase was determined by an assay procedure described in the legend of Figure 1. As shown in Figure 1, the analog is a competitive inhibitor of tyrosinase activity on DOPA, with an inhibitor constant, K_i, of 3.2×10^{-3} M. Interestingly, the analog does not serve as a substrate for oxidation by the enzyme, at least as far as colored pigment is concerned. The analog does produce a yellow-brown solution on standing in the presence of oxygen (as does DOPA), but in contrast to DOPA the reaction is extremely slow and requires elevated temperatures.

(2) E. H. Lansford, Jr., and W. Shive, *Arch. Biochem. Biophys.*, **38**, 347 (1952).

(3) S. J. Norton, C. G. Skinner, and W. Shive, *J. Org. Chem.*, **26**, 1495 (1961).

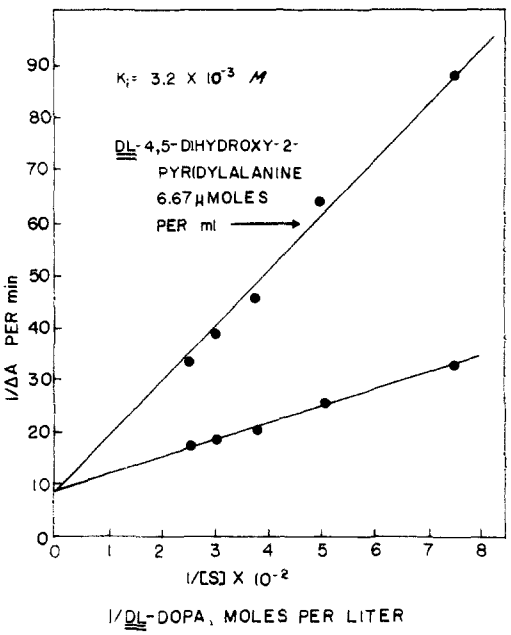


Figure 1. The reaction mixture contained in a total volume of 3 ml: DL-dihydroxyphenylalanine, amounts ranging from 4 to 12 μmoles; potassium phosphate buffer, pH 6.8, 100 μmoles; sodium ethylenediaminetetraacetate, pH 6.8, 3 μmoles; and tyrosinase preparation (Nutritional Biochemicals Corp.), 1 mg. When inhibition by the analog was studied, the reaction mixture also contained 20 μmoles of DL-4,5-dihydroxy-2-pyridylalanine. The reaction was initiated by the addition of enzyme and the rate was followed by measuring the change in absorbance (due to pigment production) at 400 mμ on a recording spectrophotometer. The temperature of the reaction mixture was 25°.

A study of the effect of DL-4,5-dihydroxy-2-pyridylalanine on the activity of DOPA decarboxylase extracted from hog kidney[4] was also conducted. The assay method was patterned after a manometric procedure for the determination of DOPA decarboxylase activity reported by Schales and Schales.[5] The analog is a substrate for the decarboxylation reaction, and the decarboxylation rate is approximately one-tenth the rate of decarboxylation of DL-DOPA. When the rates of decarboxylation were studied in reaction mixtures containing both the analog and DOPA (at concentrations not sufficient to saturate the enzyme), the resulting decarboxylation rate represented the sum of the individual decarboxylation rates. Due to the low water solubility of the analog (limit, approximately 10 μmoles/ml), concentrations of analog and DOPA sufficiently high to completely saturate the enzyme could not be obtained. It is probable that decarboxylation rates would not be additive at saturating concentrations of both substrates, since both would be competing for the same active site on the enzyme.

Experimental Section

2-Chloromethyl-5-methoxy-4H-pyran-4-one (II) was prepared by methylation of I with dimethyl sulfate according to the method of Yabuta.[6]

Ethyl 2-Acetamido-2-cyano-3-(5-methoxy-4H-pyran-4-on-2-yl)-propionate (III).—Ethyl acetamidocyanoacetate (36.1 g, 0.212 mole) was dissolved in 250 ml of dry dimethylformamide (DMF). Sodium hydride (5.08 g, 0.212 mole) was added portionwise (2 hr) with cooling and stirring. When the exothermic re-

(4) W. J. Hartman, R. L. Akawie, and W. G. Clark, *J. Biol. Chem.*, **216**, 507 (1955).

(5) O. Schales and S. S. Schales, *Arch. Biochem. Biophys.*, **24**, 83 (1949).

(6) T. Yabuta, *J. Chem. Soc.*, **125**, 575 (1924).

action was complete, 2-chloromethyl-5-methoxy-4H-pyran-4-one (II) (37.2 g, 0.212 mole), was added and the reaction mixture was stirred for 24 hr at room temperature. The DMF was distilled *in vacuo*, and the solid residue was extracted with hot $CHCl_3$-ethanol (80:20) and filtered. The filtrate was taken to dryness *in vacuo*, and the dark residue was recrystallized twice with charcoal treatment from ethanol–water. The resulting white needles weighed 33.7 g and had mp 179.5–181° (uncor).

Anal. Calcd for $C_{14}H_{16}N_2O_6$: C, 54.54; H, 5.23; N, 9.09. Found: C, 54.55; H, 4.91; N, 8.99.

DL-**4-Hydroxy-5-methoxy-2-pyridylalanine (IV).**—A mixture of 29.0 g of III and 60 g of concentrated NH_4OH was placed in a stainless steel bomb; the bomb was sealed and placed in an oven at 85–90° for 2.5 hr. The reaction mixture, a dark liquid, was then concentrated *in vacuo*, and the residual NH_4OH was removed by taking up the viscous residue in methanol and again concentrating *in vacuo*. The concentrate was ninhydrin positive (dark blue), but repeated attempts to effect crystallization were unsuccessful. Hydrolysis of this residue was effected by heating 28.0 g of the residue under reflux several hours in a suspension of 45 g of $Ba(OH)_2$ in 200 ml of water. After removal of solid $Ba(OH)_2$ by filtration, the filtrate was neutralized by the addition of CO_2 (Dry Ice). The $BaCO_3$ which precipitated was removed by filtration, and the filter cake was washed with water to remove any adsorbed amino acid. Remaining Ba^{2+} was removed from the filtrate by addition of 10% H_2SO_4 until $BaSO_4$ no longer precipitated. After filtration the filtrate was concentrated *in vacuo*, and the light brown residue was recrystallized (after decolorization by charcoal treatment) from ethanol–water to yield 14.0 g of white, hygroscopic crystalline material, mp 252–255° dec. Paper chromatography of the product with BuOH–AcOH–H_2O (4:1:1) gave a single ninhydrin spot (light brown), R_f 0.13.

Anal. Calcd for $C_9H_{12}N_2O_4 \cdot 0.5H_2O$: C, 48.87; H, 5.92; N, 12.66. Found: C, 48.78; H, 6.10; N, 12.53.

DL-**4,5-Dihydroxy-2-pyridylalanine (V).**—A solution of 1.0 g of IV and 3 ml of concentrated HI was heated under reflux for 3.5 hr. The HI was then removed *in vacuo*, and the residue was taken up in a small volume of water. The resulting solution was neutralized with concentrated NH_4OH, whereupon a precipitate of the amino acid formed (the amino acid is sparingly soluble in water). Complete removal of the amino acid from solution was effected by the addition of ethanol, and the crystalline material was collected by filtration. After decolorizing with charcoal, the amino acid was finally recrystallized from ethanol–water, and after drying overnight at 80° (vacuum desiccator, P_2O_5) weighed 0.90 g, mp 236–241° dec. Paper chromatography of the amino acid in BuOH–AcOH–H_2O (4:1:1) gave a single ninhydrin spot (light brown) with an R_f of 0.13. The amino acid reacted with $FeCl_3$ to produce a deep purple color, indicating cleavage of the methoxy group in the 5 position of the pyridine ring.

Anal. Calcd for $C_8H_{10}N_2O_4 \cdot H_2O$: C, 44.45; H, 5.60; N, 12.96. Found: C, 44.23; H, 5.58; N, 12.82.

Ultraviolet absorption spectrum showed at pH 3, λ_{max} 270 mμ, λ_{min} 256 mμ; at pH 12, λ_{max} 294–296 mμ, λ_{min} 250–252 mμ. These absorption maxima and minima were identical with those of a sample of 4,5-dihydroxy-2-hydroxymethylpyridine.

Synthetic Biologically Active Polymers. IV. N^1-Acylsulfanilamide–Formaldehyde Copolymers

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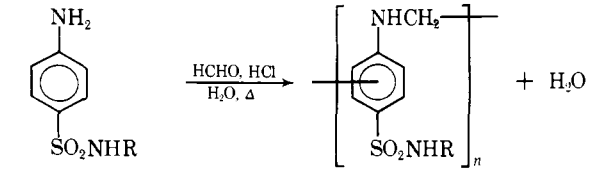
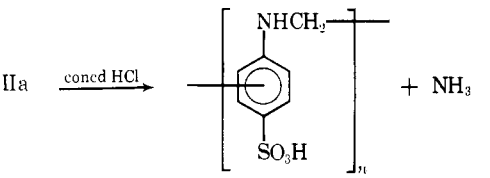
In continuing investigations aimed at attempting to discern the effect of polymerization on drugs, we had occasion to study the copolymerization of sulfanilamide (Ia), sulfabenzamide (Ib), and sulfacetamide (Ic) with formaldehyde.

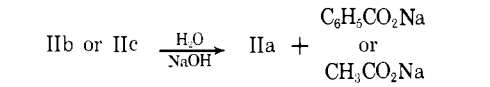


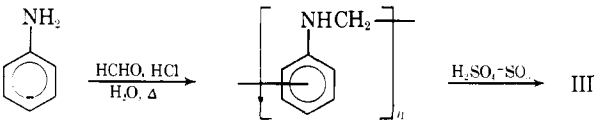
Ia, R = H
Ib, R = C_6H_5CO
Ic, R = CH_3CO

IIa, R = H
IIb, R = C_6H_5CO
IIc, R = CH_3CO



III





IV

The copolymerization processes were carried out, in general, by the method previously reported.[1a] Hydrolysis of IIa with concentrated HCl yielded III. The structure of III was previously related[1a] to the structure of a polymer obtained by the sulfonation of an aniline–formaldehyde copolymer (IV). Basic hydrolysis of both IIb and IIc yielded IIa. Acidic hydrolysis of IIa derived from both IIb and IIc yielded III. Elemental analyses and infrared spectra seem to confirm the assigned structures.

The copolymers appeared to be monodisperse. The intrinsic viscosities of IIa, IIb, and IIc in dimethyl sulfoxide (DMSO) at 25° were found to be 0.07, 0.24, and 0.10, respectively.

It was reported previously that a sulfapyridine–formaldehyde copolymer exhibited antimalarial activity above that of sulfapyridine *per se*.[1a] Therefore, copolymers IIa–c were screened for antimalarial activity employing the procedure previously reported.[1a] The screening results are summarized in Table I.

Experimental Section

Sulfanilamide–Formaldehyde Copolymer (IIa).—A mixture containing 1.67 g (0.0097 mole) of sulfanilamide (Ia), 50 ml of water, 1 ml of 4% aqueous HCl, and 1 ml of 37% aqueous formaldehyde solution was heated at reflux for 8 hr. A resin was deposited. The reaction mixture was cooled and filtered. The resin was pulverized and extracted with boiling water several times to remove unreacted monomers. The yield of dry washed product was 1.03 g (57.8%). The product softened at 187–190°. The intrinsic viscosity of the product in DMSO was 0.07; infrared data (cm^{-1}): 3400 w, 3250 s, 3100 s, 2910 m, 2800 w, 2300 w, 1650 m, 1570 s, 1490 s, 1390 w, 1290 s, 1140 s, 1080 s, 990 m, 885 w, 820 s.

Anal. Calcd for $C_7H_8N_2O_2S$: C, 45.6; H, 4.37; N, 15.20; S, 17.40. Found: C, 43.95; H, 5.03; N, 14.30; S, 16.00.

(1) For previous papers in this series see: (a) L. G. Donaruma and J. Razzano, *J. Med. Chem.*, **9**, 258 (1966); (b) R. J. Cornell and L. G. Donaruma, *J. Polymer Sci.*, **3A**, 827 (1965); *J. Med. Chem.*, **8**, 388 (1965).

(2) This work was taken in part from the thesis to be submitted by Mr. John Razzano in partial fulfillment of the requirements for the Ph.D. degree.