ethylthiourea **8** was stirred with 10% HCl (200 ml) at reflux for 30 min. The mixture was cooled to 0°, and the crude crystalline **9** was isolated by filtration. The pure compounds were obtained by recrystallization with Darco G-60 treatment. In a few cases the mercaptan was dissolved in 10% aqueous NaOH, filtered, and reprecipitated with 10% HCl before recrystallization. Table III lists the compounds prepared and their physical properties.

1-Arylimidazoles (2). The recrystallized 1-aryl-2-mercaptoimidazole (**9**) (25 g) was slurried with 20% HNO_3 (100 ml) in a 2-l. erlenmeyer flask. When this mixture was warmed on a steam bath behind a shield in the hood, a brief, vigorous reaction produced large amounts of NO_2. The mixture was kept at 100° for 5 min, before it was cooled and basified to ~pH 8 with 15% aqueous NH_3 (~400 ml).[15] The imidazole was extracted with three 25-ml portions of $CHCl_3$ and usually purified by short-path distillation or sublimation. Tables IV and V give the experimental details for these compounds.

1-(2-Carbamoylphenyl)imidazole (10a). 1-(2-Cyanophenyl)-imidazole (**2a**) (22.5 g) was dissolved in concentrated H_2SO_4 (120 ml) and heated on a steam bath for 10 min. The crude, crystalline amide was isolated by pouring the reaction mixture into ice water (500 ml), basifying to pH 8 with 28% NH_3 solution, and cooling to 0°. Recrystallization of the crude product from H_2O (120 ml) with Darco G-60 treatment gave colorless plates of **10a** (21.2 g, 81%), mp 177.5–178.5°. *Anal.* ($C_{10}H_9N_3O$) N.

1-(2-Carbamoyl-4-fluorophenyl)imidazole (10b). The above procedure gave, with 1-(2-cyano-4-fluorophenyl)imidazole (**2b**) (1.59 g) and concentrated H_2SO_4 (8 ml), after recrystallization from water (5 ml), 0.40 g (22%) of **10b**, mp 146–147°. *Anal.* ($C_{10}H_8FN_3O$) N.

1-(2-Carbamoyl-4-trifluoromethylphenyl)imidazole (10c). The above procedure gave, with 1-[2-cyano-4-(trifluoromethyl)-phenyl]imidazole (**2c**) (25.0 g) and concentrated H_2SO_4 (125 ml), after recrystallization from 33% EtOH (150 ml) with Darco G-60 treatment, 22.8 g (85%) of colorless **10c**, mp 192–193.5°. *Anal.* ($C_{11}H_8F_3N_3 \cdot H_2O$) N.

1-(2-Aminomethylphenyl)imidazole (11). An extractor thimble was charged with **2a** (42.2 g, 0.25 mole), and a THF suspension of LAH (20 g, 0.525 mole/500 ml) was heated under reflux until 2 hr after the extraction was complete. The mixture was cooled, decomposed with 20% HCl (100 ml), stripped free of THF, diluted with H_2O, filtered, and extracted with $CHCl_3$ (300 ml). The $CHCl_3$ layer was discarded, and the H_2O layer was basified with 28% NH_3 solution and extracted continuously with $CHCl_3$. The dried extracts were evaporated to leave a brown, oily residue of crude product which was distilled in a short-path still at 80–95° (0.02 mm), yield 15.55 g (36%) of **11**. A further distillation at 85° (0.1 mm) gave colorless product, mp 49.5–52°. *Anal.* ($C_{10}H_{11}N_3$) N.

1-(2-Hydroxymethylphenyl)imidazole (12). A solution of **11** (17.0 g, 0.098 mole), in a mixture of HOAc (10 ml, 0.23 mole) and H_2O (100 ml) was cooled to 0° and treated at once with a precooled solution of $NaNO_2$[16] (6.9 g, 0.1 mole) in H_2O (25 ml). The mixture was stirred at 25° for 2 hr, then basified with 28% NH_3, and continuously extracted with $CHCl_3$. The extract was dried, concentrated, and filtered through Florisil (50 g) using 600 ml of $CHCl_3$ as the eluent. The gum left on evaporation of the $CHCl_3$ was sublimed at 130° (0.1 mm) to produce 9.20 g (54%) of **12**. After a further sublimation this product melted at 100.5–102.5°. *Anal.* ($C_{10}H_{10}N_2O$) C, H, N.

(15) NaOH will open the imidazole ring, see E. S. Schipper and A. R. Day in "Heterocyclic Compounds," Vol. 5, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1957, p 205.

(16) P. A. S. Smith and D. R. Baer, *Org. Reactions*, **11**, 157 (1960).

The Syntheses and Substrate Specificity for Mammalian Dihydroxyphenylalanine Decarboxylase of 3-, 4-, 5-, and 6-Methyl-2-hydroxyphenylalanines and the Substrate Specificity of 3-, 5-, and 6-Methyl-2,4-dihydroxyphenylalanines for the Enzyme[1]

ROGER H. BOWER AND JOHN P. LAMBOOY

Department of Biochemistry, The University of Nebraska, Omaha, Nebraska

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Nuclear monomethyl-substituted *o*-tyrosines have been prepared from the corresponding azlactones *via* the benzamidocinnamic acids and the benzoylamino acids or by direct conversion to the amino acids. The 3-, 4-, and 6-methyl-*o*-tyrosines could serve as substrates while the 5-methyl-*o*-tyrosine was inert for mammalian DOPA decarboxylase. The prevention of enzyme binding by the 5-Me group led to a study of the substrate specificity of the nuclear monomethyl-substituted 2,4-dihydroxyphenylalanines. The 3- and 6-methyl-2,4-dihydroxyphenylalanines could serve as substrates while 5-methyl-2,4-dihydroxyphenylalanine was inert for mammalian DOPA decarboxylase.

The finding by Fellman and Devlin[2] in 1958 that 2-hydroxyphenylalanine (*o*-tyrosine) occurs normally in the mammalian adrenal gland makes its metabolism of interest. Armstrong, *et al.*,[3] and Nishimura and Gjessing[4] showed that *o*-tyrosine is normally decarboxylated in the human to *o*-tyramine which in turn is oxidized to *o*-hydroxyphenylacetic acid. They found that *o*-tyramine and *o*-hydroxyphenylacetic acid are excreted in the urine of normal humans in quantities ranging from 0.3 to 1.0 μg and 0.1 to 0.4 g/g of creatinine, respectively. Since it had been shown by Blaschko[5] that *o*-tyrosine could serve as a substrate for mammalian dihydroxyphenylalanine (DOPA) decarboxylase, it seemed reasonable to suspect that this enzyme is responsible for the decarboxylation observed *in vivo*, and a study of some aspects of its substrate specificity for *o*-tyrosine appeared to be of interest.

We chose to investigate the influence of the incorporation of a single CH_3 as a steric barrier into each of the

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(2) J. H. Fellman and M. K. Devlin, *Fed. Proc.*, **17**, 218 (1958).

(3) M. D. Armstrong, K. N. Shaw, and K. S. Robinson, *J. Biol. Chem.*, **213**, 797 (1955).

(4) T. Nishimura and L. R. Gjessing, *Scand. J. Clin. Lab. Invest.*, **18**, 217 (1966).

(5) H. Blaschko, *Biochem. J.*, **44**, 268 (1949).

available sites of the ring of *o*-tyrosine on the substrate specificity of these materials for DOPA decarboxylase. It was found that the 3-, 4-, and 6-methyl-*o*-tyrosines could serve as substrates for this enzyme while 5-methyl-*o*-tyrosine was inert, serving as neither substrate nor inhibitor. That the presence of a 5-CH_3 could prevent binding to the enzyme had been observed on an earlier occasion by Lambooy[6] in a study of structural requirements of the methyl-2,4-dihydroxyphenylalanines as inhibitors for tyrosinase. The similarity between 5-methyl-*o*-tyrosine and 5-methyl-2,4-dihydroxyphenylalanine is obvious but since *o*-tyrosine is not an inhibitor of tyrosinase,[6] the influence of the methyl groups in *o*-tyrosine on the inhibition of tyrosinase could not be studied.

Extensive studies have been made on the substrate specificity of DOPA decarboxylase.[7,8] Sourkes, *et al.*,[7] had shown that 2,4-dihydroxyphenylalanine was a substrate for DOPA decarboxylase and a logical extension of our study was to investigate the substrate specificity of the three nuclear-substituted methyl-2,4-dihydroxyphenylalanines for DOPA decarboxylase. It was found that the 3- and 6-methyl analogs served as substrates but that the 5-methyl-2,4-dihydroxyphenylalanine was inert, serving as neither substrate nor inhibitor for DOPA decarboxylase.

We are unable to offer an explanation as to why the 5-CH_3 in these two series of compounds prevents binding to the decarboxylase other than that it is of sufficient size and uniquely located to prevent an adequate approach to the active site of the enzyme.

The appropriate methyl-substituted *o*-methoxybenzaldehydes were converted to the azlactones by means of the Erlenmeyer reaction. The azlactones were converted to the amino acids directly utilizing P–HI. The products of these reactions were relatively insoluble and a doubt existed as to their identity. For this reason the 3-, 4-, and 5-methyl-substituted azlactones were converted to the benzamidocinnamic acids and the latter was reduced to the benzoylamino acids by means of Raney Ni catalyzed hydrogenation. The latter acids were converted to the amino acids by means of concentrated HCl in a sealed tube at 150°. The amino acids prepared by the longer procedure were identical with those prepared by the shorter procedure. The relative insolubility of the amino acids precluded the usual techniques for purification.

The 4-, 5-, and 6-methyl-*o*-tyrosines were purified by the repeated formation of the soluble Na salts and reprecipitation of the free amino acid by the addition of AcOH. This procedure resulted in destructive oxidation of the 3-methyl-*o*-tyrosine. The hydrochloride of this amino acid was purified by recrystallization from absolute EtOH–absolute Et_2O, a procedure which could not be used for the 4-, 5-, and 6-methyl-*o*-tyrosines. The properties of the new compounds are listed in Table I.

The mammalian DOPA decarboxylase was obtained from guinea pig kidney. The substrate activity was determined in a Warburg respirometer and all activities as substrate were expressed as per cent of the activity of DL-3,4-dihydroxyphenylalanine.

TABLE I

CHEMICAL AND PHYSICAL DATA ON THE INTERMEDIATES AND THE 2-HYDROXYMETHYLPHENYLALANINES

Compound	Yield, %	Mp, °C	Composition[a]
Azlactones[b]			
3-Me	61	164–165	$C_{18}H_{15}NO_3$
4-Me	51	196–197	$C_{18}H_{15}NO_3$
5-Me	45	180–181	$C_{18}H_{15}NO_3$
6-Me	49	163–168	$C_{18}H_{15}NO_3$
Acrylic acids[c]			
3-Me	87	230–231	$C_{18}H_{17}NO_4$
4-Me	98	214–215	$C_{18}H_{17}NO_4$
5-Me	89	215–216	$C_{18}H_{17}NO_4$
Propionic acids[d]			
3-Me	99	183	$C_{18}H_{19}NO_4$
4-Me	94	184–185	$C_{18}H_{19}NO_4$
5-Me	95	184–185	$C_{18}H_{19}NO_4$
Amino acids[e,h,i]			
3-Me (HCl)	87	188–190	$C_{10}H_{14}ClNO_3$
4-Me	83	248–249	$C_{10}H_{13}NO_3$
5-Me	79	248–249	$C_{10}H_{13}NO_3$
6-Me[f]	50+[g]	273–275	$C_{10}H_{13}NO_3$

[a] All compounds were analyzed for C, H, N; analytical results obtained for these elements or functions were within ±0.4% of the theoretical values. [b] Recrystallized from PhH. [c] Recrystallized from EtOH. [d] Recrystallized from 40% (v/v) AcOH. [e] See text for purification procedures. [f] Prepared by direct conversion of the azlactone. [g] The yield was subjectively thought to be like the others but lack of data prevented giving a more exact value. [h] Using the BuOH–H_2O–AcOH system, the R_f values for the 3-, 4-, 5-, and 6-Me isomers were 0.56, 0.57, 0.58, and 0.54, respectively. Using the H_2O-saturated PhOH system, the R_f values in the same order were 0.81, 0.80, 0.80, and 0.78. [i] The ir peaks (cm^{-1}) for the 3-, 4-, 5-, and 6-Me isomers were 745, 775; 735, 750, 795, 820; 715, 735, 765, 815; and 740, 775, respectively: R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1966, p 56.

Experimental Section[9]

Benzaldehydes.—2-Hydroxy-3-methylbenzaldehyde was prepared by the formylation of *o*-cresol as described by Tieman and Schotten.[10] The product (20%), bp 120–140° (15 mm), was found by gas chromatography to contain only 33% of the benzaldehyde. The benzaldehyde was purified as the bisulfite addition compound to yield, on hydrolysis, material with bp 100° (23 mm). This product was methylated with Me_2SO_4 to form 2-methoxy-3-methylbenzaldehyde (94%), bp 132° (28 mm) [lit.[11] 120° (6 mm)]. 2-Methoxy-4-methylbenzoic acid was prepared by the methylation of 4-methylsalicylic acid followed by hydrolysis. The benzoic acid was converted to the chloride and the latter was reduced by the Rosenmund reduction as described for the preparation of 3,5-dimethoxybenzaldehyde,[12] to yield 2-methoxy-4-methylbenzaldehyde (49%), bp 154° (25 mm) [lit.[13] 263–264° (atm)]. This benzaldehyde was also obtained by the methylation of 2-hydroxy-4-methylbenzaldehyde, a by-product obtained from the synthesis of 2-hydroxy-6-methylbenzaldehyde described below. *p*-Methylanisole was formylated by the use of N-methyl-N-phenylformanilide and $POCl_3$ by general procedure a, described previously,[6] to obtain 2-methoxy-5-methylbenzaldehyde (30%), bp 148° (31 mm) [lit.[13] 139° (19 mm)]. *m*-Cresol was formylated by the Tieman

(6) J. P. Lambooy, *J. Am. Chem. Soc.*, **78**, 771 (1956).
(7) T. Sourkes, P. Heneage, and Y. Trano, *Arch. Biochem. Biophys.*, **40**, 185 (1952).
(8) R. Ferrini and A. Glasser, *Biochem. Pharmacol.*, **13**, 798 (1964).
(9) Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected.
(10) F. Tieman and C. Schotten, *Ber.*, **11**, 767 (1878).
(11) R. A. Barnes and N. N. Gerber, *J. Org. Chem.*, **26**, 4540 (1961).
(12) J. P. Lambooy, *J. Am. Chem. Soc.*, **76**, 133 (1954).
(13) "Dictionary of Organic Compounds," 4th ed, Oxford University Press, New York, N. Y., 1965, p 1814.

and Schotten[10] procedure to produce a mixture of 4- and 6-methylsalicylaldehydes (42%), bp 103–110° (18 mm). The mixture of benzaldehydes was separated by the use of the aniline derivatives[14] and purified as the bisulfite addition compounds to produce 6-methylsalicylaldehyde (12%), bp 114° (22 mm) (lit.[14] mp 32°) and 4-methylsalicylaldehyde (4%), mp 57–60° (lit.[15] mp 60°). The appropriate salicylaldehydes were methylated with Me_2SO_4 to form 2-methoxy-6-methylbenzaldehyde (97%), bp 154° (25 mm), mp 40–41° (lit.[16] mp 41–42°), and 2-methoxy-4-methylbenzaldehyde (92%), bp 152° (25 mm), mp 43–44° (lit.[13] mp 42–43°). Each of the benzaldehydes was shown to be homogeneous by gas chromatography.[17]

4-(2-Methoxymethylbenzylidine)-2-phenyl-5-oxazolones.—The benzaldehydes were converted to the azlactones as described for the preparation of 4-(2,4-dimethoxymethylbenzylidine)-2-phenyl-5-oxazolones.[6] See Table I.

2-Benzamido-3-(2-methoxymethylphenyl)cinnamic Acids.—The azlactones were hydrolyzed as described for the hydrolysis of 2-benzamido-3-(2,4-dimethoxymethylphenyl)acrylic acids.[6] See Table I.

2-Benzamido-3-(2-methoxymethylphenyl)propionic Acids.—The benzamidocinnamic acids were reduced to the propionic acids as described for the preparation of 2-benzamido-3-(2,4-dimethoxyphenyl)propionic acids.[12] See Table I.

2-Hydroxymethylphenylalanines. (a) From the Corresponding Propionic Acids.—The propionic acids were converted to the amino acids by the procedure described for the preparation of 2,4-dihydroxymethylphenylalanines.[6]

(b) From the Corresponding Azlactones.—The azlactones were converted directly to the amino acids by the use of P–HI as described for the preparation of several dihydroxyphenylalanines.[6] See Table I.

The insolubility of the amino acids made them difficult to purify. Of the four amino acids, the HCl salt of only the 3-methyl-*o*-tyrosine was soluble. A solution of the hydrochloride of this amino acid was evaporated to dryness; absolute EtOH was added to the residue and this was evaporated. The resulting residue (5–10 g) was dissolved in 100 ml of absolute EtOH, treated with decolorizing charcoal, and filtered, and to the filtrate was added 200 ml of absolute Et_2O. The hydrochloride of the amino acid was obtained as a crystalline product on standing.

The 4-, 5-, and 6-methyl-*o*-tyrosines were purified as follows. Approximately 2 g was suspended in 35 ml of H_2O and when this mixture had been heated to near the boiling point, a solution of NaOH was added until all of the solid had dissolved and the solution was alkaline. Decolorizing charcoal was added, and the suspension was boiled briefly and then filtered. The filtrate was heated to the boiling point and AcOH was added dropwise until the solution was just acid to litmus. When cold, the precipitated amino acid was collected on the filter.[18] These procedures were repeated four times for the preparation of the amino acid samples for analyses and enzyme studies.

(14) O. Anselmino, *Ber.*, **50**, 395 (1917).

(15) J. C. Duff, *J. Chem. Soc.*, 547 (1941).

(16) Reference 13, p 1813.

(17) The Perkin-Elmer Model 801 gas chromatograph with a 6% DEGA column with Chromosorb support was used with helium as the carrier gas.

(18) The procedure was based on that described for the purification of tyrosine by C. J. Cox and H. King, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p 614.

The preparations of the *o*-tyrosine,[8] 2,4-dihydroxyphenylalanine,[12] and the 3-, 5-, and 6-methyl-2,4-dihydroxyphenylalanines[6] used in this study have been described on earlier occasions.

Paper Chromatography.—The R_f values were determined by the descending method on Whatman No. 1 paper using the H_2O-saturated PhOH system and also the upper phase of a BuOH–H_2O–AcOH (5:4:1) system. The amino acids were located on the paper by the ninhydrin reaction. The R_f values are given in Table I. Since the amino acids had nearly the same R_f values the ir spectra were also determined as a means of differentiating among them.

Ir spectra were determined on the amino acids dispersed in KBr pellets in a Perkin-Elmer 137 infrared spectrophotometer and are listed in Table I.

Decarboxylation Studies. The pair of kidneys were removed from a guinea pig immediately after it was killed; the kidneys were decapsulated and placed in crushed ice for 15 min. Each kidney was halved through the hilus, the pelvis, medulla and connective tissue were removed, and the combined kidney tissue was weighed. The tissue was homogenized in a 30-ml cup for a Virtis homogenizer in 4 ml of 0.067 *N* phosphate buffer (pH 6.8)/g at full speed for 1 min. The homogenate was centrifuged at 20,000*g* for 25 min in a refrigerated centrifuge to obtain the supernatant solution of the enzyme.

The substrate solutions (0.005 *M*) or buffer for the blank (2 ml) were placed in the main compartment of a Warburg flask. The enzyme solution (1 ml) and 0.05 ml of a solution of pyridoxal phosphate (100 μg/ml) were placed in one side arm and 0.5 ml of 2 *N* H_2SO_4 was placed in the other side arm. The usual technique was used to determine the μl of CO_2 produced at 37° during a 10-min period (the enzyme action was stopped and combined CO_2 was liberated by tipping in the H_2SO_4). The activity of the various amino acids as substrate for the enzyme was calculated as per cent of DOPA as standard. The results with additional data are given in Table II.

TABLE II

SUBSTRATE ACTIVITY OF THE 2-HYDROXYMETHYLPHENYLALANINES AND THE 2,4-DIHYDROXYMETHYLPHENYLALANINES FOR DOPA DECARBOXYLASE

DL-Phenylalanine	Substrate soln composn, mg/10 ml[a]	Rel[b] substrate act.
3,4-$(OH)_2$	9.85	100
2-OH	9.05	99[c] (98–100)[d]
2-OH-3-Me	9.75	95 (93–96)
2-OH-4-Me	9.75	83 (74–91)
2-OH-5-Me	9.75	0[e] (0–0)
2-OH-6-Me	9.75	31 (28–34)
2,4-$(OH)_2$	9.85	84[f] (83–85)
2,4-$(OH)_2$-3-Me	10.55	44 (43–45)
2,4-$(OH)_2$-5-Me	10.55	0[e] (–2–1)
2,4-$(OH)_2$-6-Me	10.55	5 (4–11)

[a] Equivalent to 0.005 *M*. [b] Activity of Dopa arbitrarily set at 100. [c] Reference 8 found 102%. [d] Range for three determinations. [e] Concentrations of analog to DOPA of 6:1 had no inhibitory effect. [f] Reference 8 found 88%.