mately 25°. One-centimeter quartz cells were used and the extinction coefficients per *p*-aminophenylalanine residue, ϵ, calculated from the equation

$$\epsilon = (1/cd) \log_{10} (I_0/I)$$

where I_0 is the intensity of the light emerging from the solvent, I the intensity of the light emerging from the solution, c the molar concentration of the *p*-aminophenylalanine residues, and d the thickness of the absorption cell in centimeters.

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The Syntheses, Paper Chromatography and Substrate Specificity for Tyrosinase of 2,3-, 2,4-, 2,5-, 2,6- and 3,5-Dihydroxyphenylalanines[1]

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The above amino acids have been synthesized from a variety of intermediates prepared by the Erlenmeyer reaction on the appropriate dihydroxy- or dimethoxybenzaldehydes. This required the preparation of 2,6- and 3,5-dimethoxybenzaldehyde which have now been obtained in good yields in relatively large amounts by convenient procedures. The Erlenmeyer reaction on 2,6-dimethoxybenzaldehyde produced two geometric isomeric azlactones. The paper chromatographic properties of all of the dihydroxyphenylalanines are described for a number of solvent systems, in one of which the resolution of the 2,3-, 2,4-, 2,5- and 3,5-dihydroxy amino acids has been accomplished. The substrate specificity of these amino acids for the enzyme tyrosinase has been studied. 2,4-Dihydroxyphenylalanine is a reversible inhibitor of tyrosine.

As a direct result of the observation of the toxic effect of 2,5-dihydroxyphenylalanine on *Escherichia coli* (ATCC 9637) made by Volcani,[2] and because of our interest in the chromatography and enzymatic specificity shown by the amino acids, the syntheses of 2,3-, 2,6- and 3,5-dihydroxyphenylalanines was undertaken. Since these studies also required 2,4- and 2,5-dihydroxyphenylalanine, these were also synthesized, the former from three intermediates which had not been used previously in its synthesis, and the latter from 5-acetoxy-2-hydroxybenzaldehyde, a new aldehyde not previously known. 3,4-Dihydroxyphenylalanine was available from commercial sources. Samples of these dihydroxyphenylalanines have been sent to Dr. Volcani for a continuation of his studies of amino acid metabolism in bacteria.

Barltrop[3] condensed 2-hydroxy-3-methoxybenzaldehyde with benzoylglycine, acetic anhydride and sodium acetate to obtain a product which melted at 203°. He mistakenly assumed this product to be 4-(2-hydroxy-3-methoxybenzylidene)-2-phenyl-5-oxazolone on the basis of its light yellow color. The reaction actually leads to the formation of two compounds, 4-(2-acetoxy-3-methoxybenzylidene)-2-phenyl-5-oxazolone and 3-benzamido-8-methoxycoumarin. If one attempts to purify the product by recrystallization from acetic acid as Barltrop did, it is very difficult to obtain the coumarin in pure form. Fractional crystallization of the products of the reaction from benzene produces the coumarin as white crystals melting at 207–208°. Barltrop's procedure is the best one we have found for the preparation of the coumarin.

Clemo and Duxbury[4] reported their results of the above reaction on 2-hydroxy-3-methoxybenzaldehyde in which they obtained a product referred to as 4-(2-acetoxy-3-methoxybenzylidene)-2-phenyl-5-oxazolone, obtained by recrystallization from ethanol and which melted at 156–157°. If the reaction product is fractionated from benzene one does obtain the above azlactone, but in its pure form it melts at 171–172°, and the composition of the mixture of azlactone and coumarin varies depending on whether one uses 2-hydroxy-3-methoxybenzaldehyde or 2-acetoxy-3-methoxybenzaldehyde, and on the heating period. These details are presented in the Experimental section.

Clemo and Duxbury were able to convert their mixture of azlactone and coumarin to 2,3-dihydroxyphenylalanine with no difficulty since both result in the same end-product under the conditions of the reaction used. They obtained a 71% yield of amino acid which melted at 265°. Starting with pure 4-(2-acetoxy-3-methoxybenzylidene)-2-phenyl-5-oxazolone, 3-benzamido-8-methoxycoumarin and 4-(2,3-dimethoxybenzylidene)-2-phenyl-5-oxazolone, we obtained yields of 82, 90 and 87%, respectively, of material of comparable purity. When the amino acid is pure these yields are reduced to 55, 66 and 55%, respectively, of material which melted at 280° dec.

Both Hirai[5] and Deulofeu[6] have reported the synthesis of 2,4-dihydroxyphenylalanine from 5-(2,4-dimethoxybenzylidene)-hydantoin. Hirai converted the hydantoin directly to the amino acid by means of hydriodic acid and red phosphorus to obtain a product in 47% yield which melted at 223–224°. Deulofeu converted the benzylidene hydantoin to the amino acid by a longer route involving sodium amalgam reduction and finally hydriodic acid hydrolysis. He did not report his yield but stated his product was the same as Hirai's and that it melted at 223–224°. We have synthesized 2,4-dihydroxyphenylalanine from 4-(2,4-diacetoxybenzylidene)-2-phenyl-5-oxazolone, 7-acetoxy-3-benzamidocoumarin and 2-benzamido-3-(2,4-dimethoxyphenyl)-propionic acid in yields of 16, 50 and 52%, respectively, and in all cases, when the product is pure it melts at 260–262° dec.

(1) Supported in part by a Grant-in-Aid from the American Medical Association, Council on Pharmacy and Chemistry, Grant number 659.

(2) B. E. Volcani, *J. Biol. Chem.*, **192**, 543 (1951).

(3) J. A. Barltrop, *J. Chem. Soc.*, 958 (1946).

(4) G. R. Clemo and F. K. Duxbury, *ibid.*, 1795 (1950).

(5) K. Hirai, *Biochem. Z.*, **177**, 449 (1926).

(6) V. Deulofeu, *Ber.*, **69**, 2456 (1936), and a report of the same work in *Rev. orasil. chem., Sao Paulo*, **10**, 389 (1940).

In the case of the dimethoxy azlactone the steps in the conversion were: alkaline hydrolysis yielding 89% of the acrylic acid, catalytic reduction yielding 98% of the propionic acid and hydrolysis of the methoxy groups and the benzamido group by means of concentrated hydrochloric acid in a sealed tube to produce the amino acid.

In previous work on the synthesis of 2,5-dihydroxyphenylalanine it was observed that if crude 2,5-diacetoxybenzaldehyde were recrystallized from boiling 95% alcohol prior to complete drying a new aldehyde was formed. This aldehyde has been identified as 5-acetoxy-2-hydroxybenzaldehyde and the procedure outlined in the Experimental section is a convenient method for its synthesis. This aldehyde was used for the synthesis of 2,5-dihydroxyphenylalanine by essentially the procedure already described.[7]

The synthesis of 2,6-dihydroxyphenylalanine required the preparation of 2,6-dimethoxybenzaldehyde. This material has been conveniently prepared in yields of 65% from mole lots of *m*-dimethoxybenzene by an extension of Wittig's[8] procedure. The aldehyde was converted into two geometric isomers of 4-(2,6-dimethoxybenzylidene)-2-phenyl-5-oxazolone. The predominant form (designated azlactone I) was obtained in 51% yield and the other form (designated azlactone II) in 10% yield. The formation of such geometric isomers has been previously observed by Carter[9] and coworkers in two other cases of similar azlactones. Azlactone II was convertible into azlactone I by means of pyridine but azlactone I was unaltered by such treatment.

The azlactones were hydrolyzed to their respective 2-benzamido-3-(2,6-dimethoxyphenyl)-acrylic acids (designated acrylic acid I and acrylic acid II) in excellent yields. The acrylic acids were both catalytically reduced to the same 2-benzamido-3-(2,6-dimethoxyphenyl)-propionic acid. The propionic acid was converted to 2,6-dihydroxyphenylalanine by means of concentrated hydrochloric acid in a sealed tube.

Mauthner[10] has prepared 3,5-dimethoxybenzaldehyde in poor yield by the series of reactions: 3,5-dimethoxybenzoic acid, -benzoyl chloride, -benzamide, -benzyl alcohol to the aldehyde. Later, Mauthner[11] applied the Rosenmund reduction to 5 g. of 3,5-dimethoxybenzoyl chloride to produce 3,5-dimethoxybenzaldehyde but he did not record his yield and the melting point of his product was low. We have used the Rosenmund reduction on the benzoyl chloride obtained from 0.3-mole lots of 3,5-dimethoxybenzoic acid to produce the aldehyde in 82% yield.

Recently Bailey, Bates, Ing and Warne[12] have reported the synthesis of 3,5-dihydroxyphenylalanine by the series of reactions: 3,5-dimethoxybenzoic acid, 3,5-dimethoxybenzoyl chloride, ethyl-3,5-dimethoxybenzoate, 3,5-dimethoxybenzyl alcohol, 3,5-dimethoxybenzyl chloride, ethyl acetamido-3,5-dimethoxybenzylmalonate and 3,5-dimethoxyphenylalanine to 3,5-dihydroxyphenylalanine. The decomposition point of the amino acid was not reported.

We converted the 3,5-dimethoxybenzaldehyde to 4-(3,5-dimethoxybenzylidene)-2-phenyl-5-oxazolone in 77% yield. This azlactone is readily converted in yields of 72% to the amino acid which melted at 312° dec.

The paper chromatographic properties of the amino acids were determined by conventional methods in the solvent systems, phenol–water, butyl alcohol–water–acetic acid, isobutyric acid–water and collidine–lutidine–water. Noteworthy among the results of the chromatographic studies was the observation that the 2,3-, 2,4-, 2,5- and 3,5-dihydroxyphenylalanines are resolved into their D- and L-isomers by the butanol–water–acetic acid system. Such resolution has been recently shown and proved by Dalgliesh[13] for the 2,3- and 2,5-dihydroxyphenylalanines. We have confirmed his observations on these two amino acids and also that 3,4-dihydroxyphenylalanine is not resolved by moderately long chromatograms. Chromatograms whose front moved more than 3 meters also failed to resolve the 3,4-dihydroxyphenylalanine, and while the 2,6-dihydroxyphenylalanine was also not resolved in this distance, the hour-glass shape of the spot showed that resolution was taking place.

The ability of the dihydroxyphenylalanines to serve as substrate for tyrosinase from potato was investigated. Of the six amino acids the 2,3-, 2,5- and the 3,4-acids are able to serve as substrate. It was found that 2,4-dihydroxyphenylalanine functioned as an inhibitor of tyrosine for tyrosinase. The inhibition can be reversed by means of additional tyrosine. The 2,6- and 3,5-dihydroxyphenylalanines served as neither substrates nor inhibitors.

Experimental

A. 2,3-Dihydroxyphenylalanine. Purification of Practical Grade 2-Hydroxy-3-methoxybenzaldehyde.—Practical grade 2-hydroxy-3-methoxybenzaldehyde,[14] 50 g., was dissolved in 100 ml. of ether. Sodium bisulfite, 40 g. in 100 ml. of water, was added and shaken vigorously. The product was filtered, washed on the filter with 50 ml. of ether, resuspended in 150 ml. of ether, filtered and air dried. The bisulfite addition compound suspended in 200 ml. of water and 100 ml. of 37% hydrochloric acid added with vigorous stirring, liberated the aldehyde which rapidly crystallizes. The filtered product was resuspended in 200 ml., filtered and washed on the filter with an additional 100 ml. of water, to yield 44 g. (88%) of material which melted at 46–47°.[15]

2-Acetoxy-3-methoxybenzaldehyde.—2-Hydroxy-3-methoxybenzaldehyde, 50 g. (0.329 mole), was dissolved in 51 ml. of pyridine and 34.5 ml. of acetic anhydride added. The mixture was permitted to stand 24 hours and poured into 1200 ml. of ice-water. The crystalline product was washed on the filter with 250 ml. of 1% hyrochloric acid and 150 ml. of water to yield 60.2 g. (94%) of material which melted at 76–77°.

4-(2-Acetoxy-3-methoxybenzylidene)-2-phenyl-5-oxazolone and 3-Benzamido-8-methoxycoumarin. (a) From 2-

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(8) G. Wittig, *Angew. Chem.*, **53**, 241 (1940).

(9) H. E. Carter and W. C. Risser, *J. Biol. Chem.*, **139**, 225 (1941); H. E. Carter and C. M. Stevens, *ibid.*, **133**, 117 (1940).

(10) F. Mauthner, *J. prakt. Chem.*, [a] **87**, 403 (1913); (b) **100**, 177 (1920).

(11) F. Mauthner, *ibid.*, **100**, 180 (1920).

(12) A. S. Bailey, D. H. Bates, H. R. Ing and M. A. Warne, *J. Chem. Soc.*, 4535 (1952).

(13) C. E. Dalgliesh, *ibid.*, 3940 (1952).

(14) Distillation Products Industries.

(15) All melting and decomposition points were determined on calibrated thermometers. Decomposition points were determined in an apparatus heated at a uniform rate and have been reproduced to within one or two degrees after an interval of as long as four years.

TABLE I

R_f VALUES FOR THE DIHYDROXYPHENYLALANINES IN VARIOUS SOLVENT SYSTEMS

Solvent systems	Dihydroxyphenylalanines 2,3-	2,4-	2,5-	2,6-	3,4-	3,5-	Front, cm.	Amino acid	Resolution in BuOH–H_2O–HOAc R_f L	DL	D	Front, cm.	Apparent L/D[15]
BuOH–H_2O–HOAc	0.27	0.26	0.25	0.33	0.21	0.22	35.0	2,3-	0.303		0.317	176	0.97
Isobutyric acid–H_2O	.49	.44	.43	.48	.42	.39	27.0	2,4-		0.298[e]		176	
Phenol–H_2O	.48	.37	.41	.48	.38	.27	35.5	2,5-	.274		.293	176	.94
Collidine–lutidine–H_2O	(.51)[a]	.58	(.85)[b]	.66	(.51)[c]	.64	30.6[d]	2,6-		.334[e]		176	
BuOH–H_2O	.09		.07				30.0	3,4-		.260		176	
								3,5-	.258		.272	176	.95
								2,4-	.293		.307	316	.96
								2,6-		.340[f]		341	
								3,4-		.280[g]		341	

[a] Negative ninhydrin. Spots observed under ultraviolet light two years after run. [b] Negative ninhydrin. Spots became yellow two months after run. [c] Usually completely decomposed. [d] This front distance does not apply to a, b, and c. [e] The spot was much elongated but resolution had not been accomplished. [f] The spot was not only elongated but showed a tendency toward hour-glass shape, suggesting that still longer chromatograms would resolve the amino acid. The R_f values for the centers of the enlargements were 0.334 and 0.345 for the L- and D-forms, respectively, with a L/D of 0.97. [g] A spot of small size and regular shape with no suggestion of resolution.

Hydroxy-3-methoxybenzaldehyde.—2-Hydroxy-3-methoxybenzaldehyde, 15.2 g. (0.1 mole), 18.2 g. (0.102 mole) of benzoylglycine, 11.2 g. (0.136 mole) of freshly fused sodium acetate and 44 g. (0.43 mole) of acetic anhydride were heated on the steam-bath for 3/4 hour. As much acetic anhydride as could be conveniently distilled was removed under reduced pressure and the product permitted to stand several hours. Water, 250 ml., was added, the product suspended and permitted to stand 6 to 12 hours, filtered and washed on the filter with water. The precipitate was resuspended in 150 ml. of ether, filtered and washed on the filter with 50 ml. of ether to produce 22 g. of material which melted 152–165°. The product was fractionally crystallized from benzene to produce 7.8 g. (23%) of the azlactone melting at 170–171°, and 5.4 g. (18%) of the coumarin melting 207–208°. If the procedure is followed but the heating period increased to 2 hours,[3] one obtains 22.5 g. of a mixture melting 158–165°, which on fractionation yields 7.5 g. (22%) of the azlactone melting 169–170° and 9.4 g. (32%) of the coumarin melting 208–209°. (This is the best procedure for the preparation of the coumarin.) When the heating time is increased to 4 hours, one obtains 20.5 g. of a mixture which yielded 4.5 g. (14%) of azlactone and 9.3 g. (31%) of the coumarin, indicating the harmful effects of prolonged heating on azlactone synthesis.

(b) From **2-Acetoxy-3-methoxybenzaldehyde.**—2-Acetoxy-3-methoxybenzaldehyde, 19.4 g. (0.1 mole) and the same quantities of the other components as used in the preceding reaction under the same conditions (3/4-hour heating period) yielded 21.1 g. of a mixture melting 155–160°. Fractionation produced 15.8 g. (47%) of azlactone melting 171–172° and 1.6 g. (5%) of coumarin melting 206–207°. (This is the best procedure for the preparation of the azlactone.)

Anal. Calcd. for $C_{19}H_{15}NO_5$ (azlactone): C, 67.6; H, 4.5; N, 4.15. Found: C, 68.0; H, 4.5; N, 4.15; m.p. 171–172°. Calcd. for $C_{17}H_{13}NO_4$ (coumarin): C, 69.1; H, 4.4; N, 4.74. Found: C, 69.2; H, 4.5; N, 4.6; m.p. 207–208°.

If the same procedure was followed but the material heated on the steam-bath for 2 hours we obtained 29.5 g. of a mixture which fractionated into 12.9 g. (38%) of azlactone melting 168–169° and 4.3 g. (15%) of coumarin melting 206–207°.

Heating 3.3 g. of pure azlactone, 20 ml. of acetic anhydride and 2.5 g. of freshly fused sodium acetate on the steam-bath for 3 hours resulted in the recovery of 3.0 g. of pure azlactone with no evidence of the presence of coumarin. The azlactone does not appear to be converted into the coumarin under these circumstances.

4-(2,3-Dimethoxybenzylidene)-2-phenyl-5-oxazolone.—Practical grade 2,3-dimethoxybenzaldehyde was purified by vacuum fractional distillation and recrystallization from *n*-hexane. Thus, 100 g. of the practical grade yielded 66.1 g. of pure aldehyde melting at 52–53°. 2,3-Dimethoxybenzaldehyde, 33.2 g. (0.2 mole), 36.6 g. (0.204 mole) of benzoylglycine, 16.2 g. (0.197 mole) of freshly fused sodium acetate and 200 ml. of acetic anhydride were heated on the steam-bath for 3/4 hour and processed as above. When recrystallized from benzene the yield was 42 g. (68%) of yellow needles melting 168–171°.

2,3-Dihydroxyphenylalanine. (a) From **4-(2-Acetoxy-3-methoxybenzylidene)-2-phenyl-5-oxazolone.**—Ten grams of the azlactone, 60 ml. of glacial acetic acid, 60 ml. of hydriodic acid (sp. gr. 1.7) and 6 g. of red phosphorus were heated under reflux and a stream of hydrogen for 2.5 hours. The hot mixture was filtered through asbestos and evaporated to dryness under reduced pressure. The residue was treated with 100 ml. of water and evaporated as before. The residue was suspended in 100 ml. of water at 50°, filtered and extracted three times with 50-ml. portions of ether. The water solution was concentrated to 50 ml., a layer of *n*-hexane added followed by an excess of ammonia solution. The mixture was evaporated to dryness and the residue suspended in 50 ml. of water, filtered and recrystallized from water containing a little sulfur dioxide to yield 4.8 g. (82%) of almost pure white prisms. Repeated recrystallizations yielded 3.2 g. (55%) of large white prisms which melted at 280° dec.[16]

Anal. Calcd. for $C_9H_{11}NO_4$: C, 54.8; H, 5.6; N, 7.1. Found: C, 55.0; H, 5.8; N, 7.0.

(b) From **3-Benzamido-8-methoxycoumarin.**—Five grams of the coumarin, 30 ml. of glacial acetic acid, 30 ml. of hydriodic acid (sp. gr. 1.7) and 1.5 g. of red phosphorus when treated as above yielded 3.0 g. (90%) of the amino acid. When recrystallized to a sufficiently pure state the yield was 2.2 g. (66%) of material melting at 279° dec.

(c) From **4-(2,3-Dimethoxybenzylidene)-2-phenyl-5-oxazolone.**—This azlactone, 6.18 g. (0.02 mole), 37 ml. of glacial acetic acid and 30 ml. of hydriodic acid (sp. gr. 1.7) and 1.9 g. of red phosphorus, when treated as above produced 3.44 g. (87%) of amino acid melting at 267°. When recrystallized as above the yield was 2.16 g. (55%) of material melting at 279–280° dec. Mixtures of the products of the above reactions showed no depression of melting point.

B. **2,4-Dihydroxyphenylalanine. 4-(2,4-Diacetoxybenzylidene)-2-phenyl-5-oxazolone and 7-Acetoxy-3-benzamidocoumarin.**—2,4-Dihydroxybenzaldehyde, 55.2 g. (0.4 mole), 72.8 g. (0.406 mole) of benzoylglycine, 44.8 g. (0.547 mole) of freshly fused sodium acetate and 200 ml. of acetic anhydride were heated on the steam-bath for 3/4 hour. Some of the excess acetic anhydride was distilled off under reduced pressure and the residue permitted to stand for about 2 hours. The residue was suspended in 500 ml. of water and permitted to stand for 24 hours. After filtration and washing with water the product was air dried, and then resuspended in 250 ml. of ether. When filtered it was washed twice on the filter with 50-ml. portions of ether. This produced 100 g. of dry mixed azlactone and coumarin.

The ether solution was concentrated to 100 ml. and on refrigeration produced 0.6 g. of material which was almost entirely coumarin. The ether solution was then concentrated to 25 ml. and one volume of alcohol added. On refrigeration this produced 3.1 g. of a mixture of the two

(16) Reference 4 reports the m.p. of this amino acid as 265°.

products. The 103.1 g. of the mixture was subjected to fractional crystallization from benzene and after 97 recrystallizations we obtained 41.9 g. (29%) of the azlactone as yellow needles melting (different batches) between 136 and 140°, and 30.2 g. (24%) of the coumarin as white needles melting at 190–191°. The pure azlactone has an m.p. 140–141°, the pure coumarin at 192°.

Anal. Calcd. for $C_{20}H_{15}NO_6$ (azlactone): C, 65.8; H, 4.1; N, 3.8. Found: C, 66.0; H, 4.3; N, 3.9. Calcd. for $C_{18}H_{13}NO_5$ (coumarin): C, 66.9; H, 4.1; N, 4.3. Found: C, 66.9; H, 4.2; N, 4.3.

4-(2,4-Dimethoxybenzylidene)-2-phenyl-5-oxazolone.—2,4-Dihydroxybenzaldehyde, 40 g. (0.29 mole), was converted to 2,4-dimethoxybenzaldehyde by the procedure described by Cullinane and Philpott[17] to produce 35.4 g. (74%) of material melting at 70–71°.

2,4-Dimethoxybenzaldehyde, 22.6 g. (0.136 mole), 27.3 g. (0.154 mole) of benzoylglycine, 16.8 g. (0.205 mole) of freshly fused sodium acetate and 100 ml. of acetic anhydride were heated over an open flame until all was in solution and then on a steam-bath so that the total heating time was $^3/_4$ hour. The material was processed essentially as described for the preparation of the 4-(2,3-dimethoxybenzylidene)-2-phenyl-5-oxazolone except that due to conflicting reports relative to the melting point of the 4-(2,4-dimethoxybenzylidene)-2-phenyl-5-oxazolone,[18] the material was first recrystallized from alcohol to yield 32.1 g. (77%) of material melting at 169–180°. This was recrystallized from benzene, ethyl acetate and alcohol repeatedly and finally yielded 22.5 g. (54%) of material, all batches of which melted between 173 and 178°.

2-Benzamido-3-(2,4-dimethoxyphenyl)-acrylic Acid.—4-(2,4-Dimethoxybenzylidene)-2-phenyl-5-oxazolone, 5.00 g. (0.0162 mole), was dissolved in 65 ml. of ethanol and heated to 90° and 37.5 ml. of 0.5 *N* sodium hydroxide was added. The mixture was held at 90° for 20 minutes and then cooled in an ice-bath and acidified with 5 *N* hydrochloric acid. After standing in the ice-bath for $^1/_2$ hour the product was filtered to yield 4.86 g. (92%) of white crystals melting at 223° dec. This material was recrystallized from a 50% alcohol solution (110 ml.) to yield 4.72 g. (89%) of white needles melting at 229–230° dec.[19]

2-Benzamido-3-(2,4-dimethoxyphenyl)-propionic Acid.—The preceding acrylic acid, 8.34 g. (0.0255 mole), was dissolved in 138 ml. of 0.3 *N* sodium hydroxide and 2.4 g. of Raney nickel was added. The mixture was shaken at 60 p.s.i. of hydrogen for 2 hours. Following filtration, cooling and acidification the yield was 8.82 g. (105%) of material melting 167–169°. Recrystallization from 30% (v./v.) acetic acid yielded 8.17 g. (98%) of white needles melting at 169°.[20]

2,4-Dihydroxyphenylalanine. (a) From **4-(2,4-Diacetoxybenzylidene)-2-phenyl-5-oxazolone.**—This azlactone, 7.1 g. (0.0195 mole), 29 ml. of glacial acetic acid, 22 ml. of redistilled hydriodic acid (sp. gr. 1.7) and 2 g. of red phosphorus were heated under reflux under a stream of hydrogen for 2 hours. Following filtration the solution was a deep brownish red color and on evaporation some tar and a considerable amount of free iodine was produced. Following the ether extraction the solution was much darker than in any other of our preparations involving this procedure. Following the addition of the ammonia the product was evaporated to dryness and redissolved in 25 ml. of water. Refrigeration produced tan colored crystals which, when recrystallized from water containing a small amount of sulfur dioxide yielded 0.60 g. (16%) of the amino acid as white prisms melting 255–257° dec.

(b) From **7-Acetoxy-3-benzamidocoumarin.**—This coumarin, 5.00 g. (0.0155 mole), 30 ml. of glacial acetic acid, 30 ml. of redistilled hydriodic acid (sp. gr. 1.7) and 1.5 g. of red phosphorus were heated under reflux for 2.5 hours under a stream of hydrogen. The solution was cooled and 60 ml. of water was added. After filtration the solution was evaporated to dryness and processed in the usual fashion. The crude amino acid was recrystallized from water containing a little sulfur dioxide to yield 1.52 g. (50%) of white prisms melting 260–262° dec.

(c) From **2-Benzamido-3-(2,4-dimethoxyphenyl)-propionic Acid.**—The above propionic acid, 2.00 g. (0.0061 mole) and 20 ml. of 37% hydrochloric acid was heated in a sealed tube at 150–160° for 2 hours. The reaction product was washed into a separatory funnel with 100 ml. of water and the mixture of amino acid hydrochloride solution and large needles of benzoic acid was extracted three times with 50-ml. portions of ether. The water solution was evaporated to dryness under reduced pressure and redissolved in 50 ml. of water, decolorized and then neutralized to *p*H 7.0 with ammonia solution. The solution was evaporated to dryness. The product was dissolved in 10 ml. of water, decolorized again and refrigerated to yield 0.62 g. (52%) of the amino acid as white prisms melting at 260° dec.[21]

Anal. Calcd. for $C_9H_{11}NO_4$: N, 7.1. Found: N, 7.0.

Mixed melting points of these three products all melted between 256–258° dec.

C. 2,5-Dihydroxyphenylalanine. 5-Acetoxy-2-hydroxybenzaldehyde.—In an effort to recover a number of residues of 2,5-diacetoxybenzaldehyde (m.p. 71–72°) their ethanol solutions were combined and concentrated by boiling and water was added prior to refrigeration. The crystalline product was made up of long white needles very different in appearance from the starting material. Recrystallization of this product from ethanol produced material melting at 80–81°. Ferric chloride tests showed 2,5-diacetoxybenzaldehyde to give a negative test, 2,5-dihydroxybenzaldehyde gave a blue-green test, *m*-hydroxybenzaldehyde gave a negative test, *m*-cresol gave a negative test, *o*-hydroxybenzaldehyde gave a violet test and the compound in question gave a blue-red test. It was concluded that one of the acetyl groups had been hydrolyzed by this mild procedure.

2,5-Dihydroxybenzaldehyde, 34.5 g. (0.25 mole) in 75 ml. of dry pyridine, was cooled to −10° and 50 ml. (0.53 mole) of acetic anhydride was added over a period of 15 minutes, the temperature not exceeding 0°. The mixture was allowed to stand in the ice-bath for one hour and then permitted to come to room temperature. The product was poured into 800 ml. of ice-water, filtered and while still damp, dissolved by boiling in ethanol, decolorized and refrigerated. The first batch of crystalline material melted 79–81°. Three volumes of water was added to the filtrate causing the precipitation of material which melted 71–76°. The combined lots were recrystallized from boiling ethanol to yield 31 g. (68%) of the 5-acetoxy-2-hydroxybenzaldehyde melting at 80–81°.

Anal. Calcd. for $C_9H_8O_4$: C, 60.0; H, 4.5. Found: C, 60.0; H, 4.3.

This aldehyde sublimes at 57° at 3 mm. pressure and at just above 100° at atmospheric pressure to form prisms. A labile acetyl determination was done by Neuberger's procedure.[22]

Anal. Calcd. for 23.9% labile acetyl. Found: 23.0%.

5-Acetoxy-2-hydroxybenzaldehyde, 5.5 g., was acetylated as above in 12 ml. of pyridine with 8 ml. of acetic anhydride. The crude 2,5-diacetoxybenzaldehyde was completely dried and recrystallized from ligroin to produce 5.8 g. (86%) of product melting 70–71°.

2,5-Diacetoxybenzaldehyde, 4.8 g., was dissolved in 75 ml. of 65% alcohol to which had been added one drop each of pyridine and acetic acid. The solution was refluxed for two hours during which time the solution became light yellow in color. When refrigerated a nearly quantitative yield of 5-acetoxy-2-hydroxybenzaldehyde was obtained.

The suggestive evidence of the ferric chloride test was confirmed by the infrared absorption spectra of *o*-hydroxybenzaldehyde, *m*-hydroxybenzaldehyde and 5-acetoxy-2-hydroxybenzaldehyde, which showed maximum absorptions for the hydroxyl group at 3.19, 3.02 and 3.18 μ, respectively.

4-(2,5-Diacetoxybenzylidene)-2-phenyl-5-oxazolone.—5-Acetoxy-2-hydroxybenzaldehyde, 23.1 g. (0.128 mole), 23 g. (0.129 mole) of benzoylglycine, 14.3 g. of freshly fused sodium acetate and 54 ml. of acetic anhydride was processed

(17) N. M. Cullinane and D. Philpott, *J. Chem. Soc.*, 1763 (1929).

(18) R. Pschorr and G. Knöffler, *Ann.*, **382**, 55 (1911), report the m.p. as 182° and give no yield. V. Deulofeu, *Anal. soc. espan. fis. quim.*, **32**, 152 (1934), reported softening at 167 and m.p. at 181°. P. C. Mitter and S. S. Maitra, *J. Indian Chem. Soc.*, **13**, 236 (1936), report an m.p. of 168°.

(19) Reference 18, V. Deulofeu reports the m.p. as 214°.

(20) Reference 19 reported a yield of 62% of material melting 162° produced by sodium amalgam reduction.

(21) References 5 and 6 both report that the amino acid melts at 228–224°, and neither reported his yield.

(22) A. Neuberger, *Biochem. J.*, **43**, 608 (1948).

as described before,[7] to yield 29.1 g. (62%) of crude product melting 148–190°.

2,5-Dihydroxyphenylalanine.—The above crude azlactone, 70.5 ml. of hydriodic acid (sp. gr. 1.7), 115 ml. of glacial acetic acid and 3 g. of red phosphorus were processed as described before,[7] to yield 9.63 g. (56%) of 2,5-dihydroxyphenylalanine melting at 257–258° dec. The triacetyl derivative prepared from this lot of amino acid melted at 155–155.5°, and when mixed with known triacetyl derivative the melting point was undepressed.

D. 2,6-Dihydroxyphenylalanine. 2,6-Dimethoxybenzaldehyde.—A two-liter, three-neck round-bottom flask was equipped with a gas inlet tube and an addition funnel in one side neck, a reflux condenser in the other and a rapidly turning, sealed stirrer in the middle neck. The paddle of the stirrer was made of pieces of twisted stainless steel to ensure scratching of the lithium metal. The apparatus was suitably protected by drying tubes and flushed with dry nitrogen for one hour. Lithium metal, 13.9 g. (2.02 moles), cut into small pieces was added to the flask through an escaping stream of nitrogen. Dry ether, 750 ml., was added, and after starting the stirrer 10 to 15 ml. of bromobenzene was added to start the reaction. During one hour a total of 157 g. (1.0 mole) of bromobenzene was added at a rate which maintained a vigorous but manageable reaction. Following the addition of the bromobenzene the contents were heated to reflux temperature by a warm water-bath for ¾ hour. Resorcinol dimethyl ether, 138 g. (1.0 mole), was added over a period of 10 to 15 minutes and the reaction mixture drawn by gentle suction into a two-liter flask equipped with an inlet tube, a condenser and an addition funnel. (This transfer was made to free the apparatus for another run and also to remove a small amount of dirt.) The mixture is permitted to stand at room temperature for three days during which time large crystals are deposited, and then 135 g. (1.0 mole) of N-methylformanilide was added over a period of ¾ hour. The mixture was then refluxed for ½ hour and then poured with vigorous stirring into a mixture of 500 g. of ice and one liter of 3 *N* hydrochloric acid. The precipitate was collected by filtration, washed on the filter with 100 ml. of ether, and dried to yield 71.3 g. of white product melting at 96–97°. The water phase was extracted with ether and the combined ether solutions evaporated and the residue distilled under reduced pressure; only that material boiling between 130–140° at 13 mm. pressure being collected. This fraction is mashed in 50 ml. of ether and filtered to produce 36.8 g. of white material melting at 96–98°. All the residues were dissolved in ether and shaken with a saturated solution of sodium bisulfite. The water phase is made acid and extracted with ether. After evaporation of the ether the residue is recrystallized from cyclohexane to yield 4.2 g. of material melting 97–98°. The entire yield, 112.3 g., can be purified a little by recrystallization from cyclohexane to yield 107 g. (65%) of 2,6-dimethoxybenzaldehyde melting at 97–99°. The semicarbazone melted at 194–195°.[23]

4-(2,6-Dimethoxybenzylidene)-2-phenyl-5-oxazolone I and II.—2,6-Dimethoxybenzaldehyde, 32.8 g. (0.197 mole), 35.4 g. (0.197 mole) of benzoylglycine, 16.2 g. (0.198 mole) of freshly fused sodium acetate and 200 ml. of acetic anhydride were heated on the steam-bath for 1.5 hours. The mixture was subjected to reduced pressure and while still heated, 180 ml. of acetic anhydride was distilled off. When cold, 250 ml. of water was added, the mixture suspended and permitted to stand for 3 hours. After filtration the precipitate was resuspended in 500 ml. of ether and filtered again. The ether solution was washed with water, concentrated to 100 ml. and cooled. The precipitate was collected, the filtrate concentrated to 50 ml. and cooled to yield a small amount of material. The combined product was fractionated from ethanol to yield 31 g. (51%) of large yellow needles which melted at 121–122° (azlactone I) and 6.0 g. (10%) of fine yellow needles which melted at 168–169° (azlactone II) and 0.4 g. of unidentified material which melted at 234–236°.

Anal. Calcd. for $C_{18}H_{15}NO_4$ (azlactone I): C, 69.9; H, 4.9; N, 4.5. Found: C, 69.8; H, 4.8; N, 4.8. Calcd. for $C_{18}H_{15}NO_4$ (azlactone II): C, 69.9; H, 4.9; N, 4.5. Found: C, 70.2; H, 5.2; N, 4.4.

Azlactone II, 0.300 g. (m.p. 168–169°), was dissolved in 8.0 ml. of pyridine and permitted to stand at room temperature for 10 minutes. The solution was poured into 20 ml. of cold 2.5 *N* hydrochloric acid. The precipitate was recrystallized from ethanol to produce a nearly quantitative yield of azlactone I melting at 121–122°. When this material was mixed with known azlactone I the melting point was not depressed.

When azlactone I was treated as above, it was recovered unaltered.

2-Benzamido-3-(2,6-dimethoxyphenyl)-acrylic Acid I.—Azlactone I, 24.0 g. (0.078 mole), was dissolved in 322 ml. of ethanol on the steam-bath and 186 ml. of 0.5 *N* sodium hydroxide was added. After heating at 90–95° for 20 minutes the solution was cooled and acidified. The product was filtered, washed on the filter with water and sucked as dry as convenient. It was immediately recrystallized from ethanol to yield 24.9 g. (98%) of white prisms melting at 246–247° dec.

Anal. Calcd. for $C_{18}H_{17}NO_5$: C, 66.0; H, 5.2; N, 4.3. Found: C, 65.8; H, 5.5; N, 4.7.

2-Benzamido-3-(2,6-dimethoxyphenyl)-acrylic Acid II.—Azlactone II, 6.0 g. (0.0194 mole), 78 ml. of ethanol and 45 ml. of 0.5 *N* sodium hydroxide treated as above yielded 5.65 g. (89%) of material as fine white needles melting at 220–222° dec.

Anal. Calcd. for $C_{18}H_{17}NO_5$: C, 66.0; H, 5.2; N, 4.3. Found: C, 66.2; H, 5.5; N, 4.1.

Acrylic acid I, 0.250 g. and 5.0 ml. of acetic anhydride were heated on the steam-bath for 15 minutes. The product was poured into a mixture of ice and water, and when crystalline, filtered. After recrystallization from ethanol the yield was 0.235 g. (100%) of azlactone I melting at 120–121°. Acrylic acid II treated in the same manner yielded 0.230 g. (98%) of azlactone II melting at 166–167°.

2-Benzamido-3-(2,6-dimethoxyphenyl)-propionic Acid. (a) From Acrylic Acid I.—Acrylic acid I, 6.54 g. (0.02 mole), was dissolved in 150 ml. of 0.26 *N* sodium hydroxide; 2.4 g. of Raney nickel was added and the mixture shaken for 48 hours at room temperature at 60 p.s.i. After filtration the filtrate was cooled in an ice-bath, acidified and kept in the ice-bath for an additional 30 minutes. The product was filtered, washed with water and immediately recrystallized from 53% (v./v.) acetic acid to yield 6.42 g. (98%) of white prisms melting at 165–166°. (The compound can also be satisfactorily recrystallized from ethanol.)

Anal. Calcd. for $C_{18}H_{19}NO_5$: C, 65.6; H, 5.8; N, 4.3. Found: C, 65.2; H, 6.1; N, 4.2.

(b) From Acrylic Acid II.—Acrylic acid II, 4.00 g. (0.0122 mole), in 80 ml. of 0.26 *N* sodium hydroxide with 1.2 g. of Raney nickel added was shaken for 24 hours as above. After the same treatment as above, 3.91 g. (97%) of material melting at 165–166° was obtained. When the products from procedures a and b were mixed the m.p. was not depressed.

2,6-Dihydroxyphenylalanine.—The above propionic acid, 3.00 g. (0.00914 mole) and 25.0 ml. of 37% hydrochloric acid were heated in a sealed tube for 2 hours at 150–160°. The processing of the product of the reaction was the same as that used in the preparation of the 2,4-dihydroxyphenylalanine by the sealed tube reaction, but varied in the following respects. Following the evaporation to dryness after the adjustment to *p*H 7.0, the material was white and after triturating with 10 ml. of water it was filtered to yield 1.40 g. (79%) of material which melted 249–250° dec. This material is of sufficient purity for most purposes for which such amino acids can be used but can be purified at great cost in material by repeated recrystallizations from water to yield material as clusters of fine white prisms which melt at 263–265° dec.

Anal. Calcd. for $C_9H_{11}NO_4$: C, 54.8; H, 5.6; N, 7.1. Found: C, 54.7; H, 6.0; N, 6.9.

E. 3,5-Dihydroxyphenylalanine. 3,5-Dimethoxybenzaldehyde.—3,5-Dihydroxybenzoic acid[24] was methylated by the procedure described by Mauthner[10a] to produce 3,5-dimethoxybenzoic acid. 3,5-Dimethoxybenzoic acid, 54.6 g. (0.3 mole), was refluxed for 4 hours with 70 ml. of thionyl chloride. The excess thionyl chloride was removed at reduced pressure on the steam-bath and the product distilled under reduced pressure to produce 52.3 g. (87%) of the acid

(23) Reference 8 reported 189–190°.

(24) A. W. Weston and C. M. Suter, *Org. Syntheses*, **21**, 97 (1941).

chloride; b.p. 163–165° at 19–20 mm. pressure. Yields from 0.148 and 0.268 mole of the benzoic acid were 88 and 87%, respectively. The acid chloride was always subjected to the following reaction immediately after it had been distilled. 3,5-Dimethoxybenzoyl chloride, 52.3 g. (0.261 mole), was dissolved in 200 ml. of pure dry xylene and 10.6 g. of 5% palladium-on-barium sulfate catalyst[25] was added, followed by 0.26 ml. of catalyst poison.[26] The reaction mixture was heated in an oil-bath to cause the xylene to reflux, and stirred by a sufficiently rapid stirrer to keep the catalyst suspended. In general, the apparatus was like that described[26] except that a ground glass, Trubore Bearing[27] stirrer was used. The reaction was complete in 40 minutes as indicated by the titration of the evolved hydrogen chloride. One batch employing catalyst poison which was one week old required 2.5 hours. The catalyst was removed by filtration, washed with dry xylene and the solvent removed by flash distillation on the steam-bath. The 3,5-dimethoxybenzaldehyde was distilled under reduced pressure to yield 36.4 g. (82%) of material of b.p. 150–152° at 15–16° mm. pressure, and when recrystallized from *n*-hexane it melted at 48°.[28] Quantities of acid chloride equivalent to 0.125 and 0.231 mole produced yields of 85 and 80%, respectively.

4-(3,5-Dimethoxybenzylidene)-2-phenyl-5-oxazolone.—3,5-Dimethoxybenzaldehyde, 56.3 g. (0.339 mole), 62.6 g. (0.350 mole) of benzoylglycine, 37.7 g. (0.46 mole) of freshly fused sodium acetate and 176 ml. of acetic anhydride were heated over an open flame until all was in solution and then on the steam-bath so that the total heating time was 3/4 hour. Excess acetic anhydride was removed under reduced pressure and when cold, 250 ml. of water was added. The product was suspended and permitted to stand for 6 hours. Following filtration the product was air dried and recrystallized from benzene to yield 80.3 g. (77%) of material as yellow needles which melted at 154°.

Anal. Calcd. for $C_{18}H_{15}NO_4$: C, 69.9; H, 4.9; N, 4.5. Found: C, 70.1; H, 4.7; N, 4.4.

3,5-Dihydroxyphenylalanine.—The above azlactone, 10.0 g. (0.0324 mole), 40 ml. of glacial acetic acid, 30 ml. of hydriodic acid (sp. gr. 1.7) and 2.5 g. of red phosphorus were heated under reflux for 2.5 hours under a gentle stream of hydrogen. The hot reaction mixture was filtered through an asbestos mat and the phosphorus washed with a little hot acetic acid. The mixture was evaporated to dryness under reduced pressure and under hydrogen. Fifty milliliters of water was added and the evaporation repeated. The residue was warmed to 50° with 200 ml. of water, filtered and extracted five times with 50-ml. portions of ether. The water solution was concentrated to 100 ml., a layer of *n*-hexane added followed by an excess of ammonia solution. The material was evaporated to dryness, dissolved by boiling in 130 ml. of water containing a little sulfur dioxide, decolorized, filtered and refrigerated. The crystalline product is recrystallized from water to yield 4.6 g. (72%) of white prisms which melted at 312° dec. (uncor.).

Anal. Calcd. for $C_9H_{11}NO_4$: C, 54.8; H, 5.6; N, 7.1. Found: C, 54.5; H, 5.7; N, 7.1.

Substrate Specificity and Inhibition Studies.—The enzyme preparation was prepared similarly to that used by Raper and Wormall[29] and was essentially a potato juice. The prepared tubes were aerated for short and equal intervals of time during the course of the enzyme activity measurements. All values given have been corrected for air oxidation of the particular amino acid[30] in the absence of the enzyme and for the color produced by the action of the enzyme on various substrates introduced with the enzyme solution. All measurements were made with a Klett-Summerson photoelectric colorimeter using filter No. 54.

In the substrate studies each tube contained 3.0 ml. of *M*/10 phosphate buffer (*p*H 7.0), 0.2 mg. of the particular amino acid and 1.0 ml. of enzyme solution. The color production as indicated by the corrected galvanometer readings were as follows: the 3,4-acid gave a reading of 545 in 16 min.; the 2,5-acid a reading of 620 in 58 min.; the 2,3-, 2,6-, 3,5- and 2,4-acids gave readings of 310, 0, 0, and −70, respectively, at 250 min. The results indicated that 2,4-dihydroxyphenylalanine was inhibiting tyrosinase.

In the inhibition studies each tube contained 5.0 ml. of *M*/10 phosphate buffer (*p*H 7.0), 0.25 mg. of L(−)-tyrosine (0.5 mg./ml.), 0.1 to 1.0 mg. of 2,4-dihydroxyphenylalanine (1.0 mg./ml.), 1.0 ml. of enzyme solution and water to make 10 ml. The following readings were obtained for the designated quantities of amino acid at 250 min.: enzyme only, 62; tyrosine only, 159; 0.1 mg. 2,4-, 118; 0.2 mg. 2,4-, 90; 0.3 mg. 2,4-, 78; 0.5 mg. 2,4-, 62 and 1.0 mg. 2,4-, 52. When the 2,4-acid was added to the enzyme tubes which contained no additional tyrosine the following readings were obtained: 0.1 mg. 2,4-, 48; 0.2 mg. 2,4-, 39. The addition of larger amounts of the 2,4-acid did not inhibit color formation beyond these points. When it was observed that the tyrosinase was inhibited by the 2,4-dihydroxyphenylalanine it became of interest to learn if additional tyrosine could reverse the inhibition.

In the reversal studies each tube contained 5.0 ml. of 0.1 *M* phosphate buffer (*p*H 7.0), 0.2 mg. of 2,4-dihydroxyphenylalanine (1.0 mg./ml.), 0.25 to 1.25 mg. of L(−)-tyrosine (0.5 mg./ml.) as designated, 1.0 ml. of enzyme solution and water to make 10 ml. The following readings were obtained for the designated quantities of amino acid at 150 min.: enzyme only, 16; 0.25 mg. tyrosine but no 2,4-, 51; 0.25 mg. tyrosine, 32; 0.35 mg. tyrosine, 39; 0.5 mg. tyrosine, 54; 0.75 mg. tyrosine, 81; 1.0 mg. tyrosine, 105; and 1.25 mg. tyrosine, 132. The addition of more tyrosine caused a still greater increase in color density.

Paper Chromatography.—The R_f values of these amino acids were determined in the usual fashion and are not listed as exact and reproducible but are given to indicate the relative positions of these amino acids when run simultaneously in the described solvent systems.

The resolution of the 2,3-, 2,5- and 3,5-dihydroxyphenylalanines was observable in the butanol–water–acetic acid system by using a fast paper 30 inches long and permitting the solvent to run off of the end until the amino acids had traveled most of the length of the paper. Riboflavin was used as a marker because it could be observed under ultraviolet light and has a reproducible R_f value of 0.30 under our conditions. When the riboflavin had reached to within 3 inches of the end of the paper the chromatogram was stopped. The amino acid spots were visualized by the usual ninhydrin spray and drying at room temperature. The 2,4- and the 2,6-dihydroxyphenylalanines could not be resolved in this relatively short distance but after processing in the above fashion the paper was sewed to another 30-inch paper and the process continued. After the riboflavin had traveled 95 cm., indicating an apparent solvent front of 316 cm. the D- and L-forms of the 2,4-dihydroxyphenylalanine had become clearly resolved. A chromatogram of somewhat greater length failed to completely resolve the 2,6-dihydroxyphenylalanine but there is a strong indication that it was in the process of being resolved.

All of the dihydroxyphenylalanines were found to be stable under the conditions imposed by ordinary Whatman paper No. 1 and the butanol–water–acetic acid, and the isobutyric acid–water systems. Only when washed paper, fresh solvent made from pure phenol and a trace of hydrocyanic acid in the chamber atmosphere were used did all the amino acids survive the phenol–water system.

In all preparations of collidine–lutidine–water the 2,4-, 2,6- and 3,5-amino acids survived and were readily located. The 3,4-amino acid did not always survive and the 2,3- and the 2,5-amino acids never gave the usual ninhydrin test. Observing a two-year old chromatogram under ultraviolet light revealed spots for the 2,3-amino acid which gave R_f values of 0.51. Two months after spraying with ninhydrin a yellow spot developed in a chromatogram to reveal the location of the 2,5-amino acid or its decomposition products. Its R_f value was 0.85.

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(25) R. Mozingo, *Org. Syntheses*, **26**, 77 (1946). The catalyst was washed 15 times with distilled water by centrifugation.

(26) E. B. Hershberg and J. Cason, *ibid.*, **21**, 85 (1941). The catalyst poison must be used within 3 or 4 days of preparation.

(27) Ace Glass, Inc., Vineland, N. J., Stirrer No. 8244. During the reaction the bearing was kept wet with xylene.

(28) Reference 10b reported an m.p. of 45–46°.

(29) H. S. Raper and A. Wormall, *Biochem. J.*, **17**, 454 (1923).

(30) During a 250-minute period, air oxidation of the 3,4-acid is appreciable, that of the 2,5-acid is very moderate and that of the others is insignificant. Water solutions of the amino acids which had been allowed to stand for two months showed extensive oxidation of the 2,3- and 3,4-acids, moderate oxidation of the 2,4-, 2,5- and 2,6-acids, and no observable oxidation of the 3,5-acid.