The same acid was obtained when ester IX was hydrolyzed.

When 5 g. of acid X was heated to 180° until evolution of carbon dioxide ceased, there remained 4 g. (93%) of **5-oxo-3-phenyl-5-(3-pyridyl)-pentanoic acid** which separated from 95% ethanol in colorless needles, m.p. 147–148°.

Anal. Calcd. for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61. Found: C, 71.24; H, 5.68.

Reaction of β-Pyridyl β-Styryl Ketone with Fluorene Derivatives.—Ketone I (5.2 g.) was allowed to react with fluorene (8.4 g.) and 50% aqueous sodium hydroxide (5 ml.) in pyridine (50 ml.) for 12 hours at room temperature and the solvents were then removed under reduced pressure. Crystallization of the residue from benzene yielded 4.0 g. (55%) of **5-(9-fluoryl)-4-nicotinoyl-3,5-diphenyl-1-(3-pyridyl)-1-pentanone (XV)**, m.p. 258–260°.

Anal. Calcd. for $C_{41}H_{32}N_2O_2$: C, 84.22; H, 5.52. Found: C, 84.07; H, 5.72.

The mother liquor from crystallization of XV was evaporated and the residue was dissolved in hot 95% ethanol. Excess fluorene separated from the cooled solution. Addition of 3.0 g. of picric acid to the filtered ethanol solution produced 5.8 g. (representing 39% of XII) of the **picrate of 9-(2-nicotinoyl-1-phenylethyl)-fluorene** in yellow needles, m.p. 180–182°.

Anal. Calcd. for $C_{33}H_{24}N_4O_8$: C, 65.55; H, 4.00; N, 9.26. Found: C, 65.68; H, 3.87; N, 9.18.

When 2 g. of the picrate was shaken with 100 ml. of 3% aqueous ethanolamine there was obtained 1.0 g. of ketone XII, which melted at 105–106° after crystallization from ethanol.

Anal. Calcd. for $C_{27}H_{21}NO$: C, 86.37; H, 5.64. Found: C, 86.48; H, 5.74.

The yields of XV and XII were 11 and 62%, respectively, when this condensation was repeated with a 10-to-1 molar ratio of fluorene to ketone I.

A mixture of ketone I (5.2 g.), 9-carboethoxyfluorene (5.9 g.) and 95% ethanol (20 ml.) was warmed until all solids had dissolved and diethylamine (0.5 g.) was then added. After 2 days at room temperature the crystalline adduct was filtered. Concentration of the mother liquor afforded additional material making the yield of **9-carboethoxy-9-(2-nicotinoyl-1-phenylethyl)-fluorene (XIII)** essentially quantitative; m.p. 87–91°. Recrystallization from ethanol raised the m.p. to 91–92°.

Anal. Calcd. for $C_{30}H_{25}NO_3$: C, 80.51; H, 5.63. Found: C, 80.48; H, 5.82.

LOS ANGELES, CALIFORNIA

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, THE ARMOUR LABORATORIES]

The Preparation of Methyl-substituted DL-Phenylalanines

BY ROSS R. HERR, TAKASHI ENKOJI AND JOSEPH P. DAILEY

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A number of methyl-substituted phenylalanines and the corresponding diethyl acetamidomalonate intermediates have been prepared. The amino acids were evaluated as phenylalanine antagonists in the *E. coli* assay. The substituted malonates were prepared by the reaction between sodium diethyl acetamidomalonate and the appropriate benzyl bromide, benzyl chloride or benzyltrimethylammonium iodide. A modified procedure for the condensation of a quaternary iodide with sodium diethyl acetamidomalonate was devised. Hydrolysis and decarboxylation of these condensation products resulted in 2-methyl-, 3-methyl-, 4-methyl-, 2,3-dimethyl-, 2,4-dimethyl-, 2,5-dimethyl-, 2,6-dimethyl, 3,4-dimethyl-, 3,5-dimethyl- and 2,4,6-trimethylphenylalanines. Of these, only 3-methylphenylalanine will inhibit completely the growth of *E. coli*. Each of the compounds will reverse the growth-inhibiting effect of β-2-thienylalanine.

In view of the potential usefulness of compounds which are competitively antagonistic to specific amino acids, we have prepared a series of compounds which are chemically similar to phenylalanine. Each compound possesses the basic structure of phenylalanine with one or more methyl groups in different positions on the aromatic ring.

Previous investigators have shown that some *dl*-phenylalanines substituted in the phenyl ring act as competitive antagonists in the utilization of phenylalanine by microörganisms. Some of these compounds also are capable of replacing phenylalanine in reversing the inhibitory action of other competitive antagonists such as β-2-thienylalanine.[1-3]

The effect of methyl substituents in these phenylalanines would be expected to be more steric than polar. Information pertaining to the relationship between steric and polar effects and that between utilization and competitive antagonism could possibly be obtained by means of these methyl-substituted phenylalanines. Alkyl substitution in any position on the phenyl ring would hinder the hydroxylation of that position, and this, in turn, would block some of the possible pathways through which this amino acid could be metabolized.

Dakin[4] has prepared 4-methylphenylalanine and found it to cause a 35% inhibition of DOPA decarboxylase in dogs. In later studies,[3] it was found to reverse the inhibitory action of β-2-thienylalanine in *E. coli*. Burckhalter and Stephens[1] prepared 3-methylphenylalanine, and Harper, Furst and Morris[5] found that this compound did not inhibit the growth of *Leuconostoc mesenteroides* P-60, a lactobacillus, in concentrations up to 100 γ per cc. of medium.

The compounds listed in Table I were synthesized by condensing benzyl halides or benzyltrimethylammonium iodides with sodium diethyl acetamidomalonate, and the resulting substituted malonic esters were hydrolyzed and decarboxylated by refluxing with a halogen acid.[6]

Three methods were used for the preparation of the substituted malonic esters, and the data pertaining to these products are summarized in Table II. Method A consists of condensing diethyl acetamidomalonate with the methyl-substituted ben-

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(5) H. A. Harper, A. Furst and F. D. Morris, *Wasmann J. Biol.*, **8**, 299 (1950).

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TABLE I

PHYSICAL AND MICROBIOLOGICAL PROPERTIES OF METHYL-SUBSTITUTED PHENYLALANINES

Phenylalanine	Empirical formula	M.p., °C. dec.	Recryst. solvent	Amount required to reverse effect of 5 γ of β-2-thienylalanine on *E. coli* γ/6 cc.	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
2-Methyl (I)	$C_{10}H_{13}NO_2$	260–262	Water	10	67.0	7.31	7.82	66.8	7.39	7.64
3-Methyl (II)	$C_{10}H_{13}NO_2$	242–246	Ethanol–water	10[a]	67.0	7.31	7.82	66.8	7.53	7.82
4-Methyl (III)	$C_{10}H_{13}NO_2$	276–279	Water	2.5	67.0	7.31	7.82	67.0	7.33	7.68
2,3-Dimethyl (IV)	$C_{11}H_{15}NO_2$	244–245	Water	5	68.4	7.83	7.25	68.3	7.84	7.06
2,4-Dimethyl (V)	$C_{11}H_{15}NO_2$	244–246	Water	25	68.4	7.83	7.25	68.3	7.81	7.28
2,5-Dimethyl (VI)	$C_{11}H_{15}NO_2 \cdot H_2O$	243–245	Water	10	62.5	8.11	6.63	62.7	8.07	6.69
2,6-Dimethyl (VII)	$C_{11}H_{15}NO_2$	236–237	Acetic acid	500	68.4	7.83	7.25	68.4	7.76	7.23
3,4-Dimethyl (VIII)	$C_{11}H_{15}NO_2$	249–251	Water	2.5	68.4	7.83	7.25	68.4	7.81	7.10
3,5-Dimethyl (IX)	$C_{11}H_{15}NO_2$	245–249	Water	25	68.4	7.83	7.25	68.4	7.73	7.34
2,4,6-Trimethyl (X)	$C_{12}H_{17}NO_2$	242–244	Acetic acid	250	69.5	8.27	6.76	69.6	8.28	7.00

[a] 2000 γ/6 ml. media causes complete inhibition of growth of *E. coli*. The other methyl-substituted phenylalanines do not inhibit the growth of *E. coli* at 10,000 γ/6 ml. media.

TABLE II

PREPARATION AND PROPERTIES OF METHYL-SUBSTITUTED DIETHYL BENZYLACETAMIDOMALONATES

Diethyl benzylacetamidomalonate	Empirical formula	M.p., °C.	React. time, hr.	Yield, %	Hydrolyzing agent	Recryst. solvent	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found N	Found H
2-Methyl (Ia)	$C_{17}H_{23}NO_5$	88–89	4	70[a]	20% HBr[d,i]	Hexane	63.5	7.21	4.36	63.4	7.12	4.32
3-Methyl (IIa)	$C_{17}H_{23}NO_5$	108–109	3	47[a]	20% HBr[d,i]	Cyclohexane–hexane or 95% ethanol	63.5	7.21	4.36	63.4	7.53	4.34
4-Methyl (IIIa)	$C_{17}H_{23}NO_5$	111–113	8	73[a]	25% HCl[e,i]	Benzene–hexane	63.5	7.21	4.36	63.6	7.22	4.43
2,3-Dimethyl (IVa)	$C_{18}H_{25}NO_5$	92.5–93.5	20	63[c]	Concd. HCl[d,j]	Hexane	64.5	7.51	4.18	64.5	7.64	4.24
2,4-Dimethyl (Va)	$C_{18}H_{25}NO_5$	118–119	3	54[a]	20% HBr[d,i,j] or 25% HCl	Cyclohexane or hexane	64.5	7.51	4.18	64.7	7.54	4.27
2,5-Dimethyl (VIa)	$C_{18}H_{25}NO_5$	76–77	4	74[a]	25% HCl[f,i]	Hexane	64.5	7.51	4.18	64.8	7.62	4.51
2,6-Dimethyl (VIIa)	$C_{18}H_{25}NO_5$	99–100	20	88[c]	Concd. HCl[g,j]	Ethanol	64.5	7.51	4.18	64.4	7.73	4.28
3,4-Dimethyl (VIIIa)	$C_{18}H_{25}NO_5$	109–110	3	53[b]	Concd. HCl[d,j]	Hexane	64.5	7.51	4.18	64.7	7.49	4.47
3,5-Dimethyl (IXa)	$C_{18}H_{25}NO_5$	149–150	7	67[a]	25% HCl[h,j]	95% Ethanol	64.5	7.51	4.18	64.5	7.51	4.16
2,4,6-Trimethyl (Xa)	$C_{19}H_{27}NO_5$	81–82	11	59[a]	25% HCl[f,j]	Hexane	65.3	7.79	4.01	65.4	7.68	4.14

[a] Method A. [b] Method B. [c] Method C. [d] Hydrolysis time 6 hr. [e] Hydrolysis time 3.5 hr. [f] Hydrolysis time 4 hr. [g] Hydrolysis time 5 hr. [h] Hydrolysis time 8 hr. [i] Neutralizing agent IR-4B. [j] Neutralizing agent, concd. NH_4OH.

zyl halide, using sodium hydride as the condensing agent and toluene as the solvent.[6] The three xylyl bromides were obtained commercially. Side-chain bromination of mesitylene with N-bromosuccinimide resulted in 3,5-dimethylbenzyl bromide. Chloromethylation of *p*-xylene, *m*-xylene and mesitylene yielded 2,4-dimethyl-, 2,5-dimethyl- and 2,4,6-trimethylbenzyl chlorides, respectively.[7,8]

In Method B, a methyl-substituted benzyl chloride was condensed with diethyl acetamidomalonate in the presence of an equivalent amount of sodium ethoxide in ethanol. The starting 3,4-dimethylhexyl chloride was prepared by the chlorination of 3,4-dimethylbenzyl alcohol with thionyl chloride. The alcohol, in turn, was prepared by the reaction of the Grignard reagent from 4-bromo-*o*-xylene[9] with formaldehyde.[10]

A convenient method for the preparation of the 2,3-dimethyl- and the 2,6-dimethylbenzyl halides was not found, and the corresponding benzyl trimethylammonium iodides of these compounds were prepared. In Method C, these quaternary iodides were condensed with sodium diethyl acetamidomalonate in xylene by a procedure adapted from that used by Snyder and Smith.[11] The successive treatment of benzyltrimethylammonium iodide with sodium amide in liquid ammonia was used to prepare 2,3-dimethylbenzyltrimethylammonium iodide.[12] A Sandmeyer reaction of 2,6-dimethylaniline, using a mixture of sodium, potassium and cuprous cyanides yielded 2-cyano-*m*-xylene, which was reduced to 2,6-dimethylbenzylamine with lithium aluminum hydride. Methylation and quaternization of this amine with methyl iodide resulted in 2,6-dimethylbenzyltrimethylammonium iodide.

The methyl-substituted phenylalanines, which were prepared from the hydrolysis and decarboxylation of these condensation products, were evaluated as amino acid antagonists in the *E. coli* assay as described by Dittmer.[13] The concentrations of amino acids needed to reverse the effect of 5 γ of β-2-thienylalanine per 6 cc. of medium were also determined. These data are summarized in Table I.

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Experimental

Side-chain Bromination of Mesitylene with N-Bromosuccinimide.—A mixture of 63.0 g. (0.53 mole) of mesitylene, 89.0 g. (0.50 mole) of N-bromosuccinimide, 0.5 g. of benzoyl peroxide and 200 ml. of dry carbon tetrachloride was refluxed

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(12) W. R. Brasen and C. R. Hauser, *Org. Syntheses*, **34**, 63 (1954).

(13) K. Dittmer, G. Ellis, H. McKennis, Jr., and V. du Vigneaud *J. Biol. Chem.*, **164**, 761 (1946).

for 1 hr. under anhydrous conditions. After filtration, the solvent was removed under reduced pressure, and the residue was distilled at 10 mm. pressure. The material boiling up to 140° was redistilled at 8–10 mm., yielding 53.0 g. (53%) of 3,5-dimethylbenzyl bromide, b.p. 100–105°.

Chloromethylation of Mesitylene.—A stream of hydrogen chloride gas was bubbled through a stirred mixture of 120 g. (1.0 mole) of mesitylene, 100 g. (1.3 moles) of 38% aqueous formaldehyde and 450 ml. of concentrated hydrochloric acid for 1 hr. with the temperature maintained at 60–70°. The two layers were separated at room temperature, and after extracting the aqueous layer with ether, the organic layer and ether extracts were combined, washed with water and dried over anhydrous magnesium sulfate. After distillation of the ether, a crystalline material separated from the cooled residue. It was removed by filtration and recrystallized from *n*-hexane to give 40.0 g. (19%) of α^2,α^4-dichloropentamethylbenzene, m.p. 103–106°. The filtrate was fractionated at 16–18 mm. pressure and the material which distilled at 60–145° was redistilled at 15–16 mm. to give 46.0 g. (27%) of 2,4,6-trimethylbenzyl chloride, b.p. 125–126°.

Chlorination of 3,4-Dimethylbenzyl Alcohol.—A solution of 18.2 g. (0.15 mole) of thionyl chloride in 80 ml. of anhydrous ether was added dropwise with stirring to 9.8 g. (0.072 mole) of 3,4-dimethylbenzyl alcohol and 0.8 ml. of pyridine in 160 ml. of anhydrous ether. After 30 min., the mixture was washed with water, dried over anhydrous magnesium sulfate and distilled to remove the ether. The residue was distilled at 10 mm., and 3.8 g. (34%) of 3,4-dimethylbenzyl chloride was collected at 95–96°.[14]

2-Cyano-*m*-xylene.—A stirred mixture of 82.3 g. (0.68 mole) of 2,6-dimethylaniline in 400 ml. of 6 *N* hydrochloric acid was cooled to −5°, and a cold solution of 50.0 g. (0.73 mole) of sodium nitrite in 340 ml. of water was added dropwise. After 30 minutes, the mixture was neutralized with solid sodium bicarbonate and slowly added to a cold solution of 39.2 g. (0.80 mole) of sodium cyanide, 104.0 g. (1.6 moles) of potassium cyanide and 80.0 g. (0.89 mole) of cuprous cyanide in 500 ml. of water. After 30 minutes, the temperature was permitted to rise to 25° and maintained until the evolution of nitrogen had ceased. The mixture was steam distilled until 2 liters of distillate was collected in a well-cooled series of traps. The distillate was extracted with ether and washed with 2 *N* sodium hydroxide and water. After drying over anhydrous magnesium sulfate, the ether was removed by distillation and the residue crystallized upon cooling. The solid was recrystallized from *n*-hexane; yield 64.6 g. (66%), m.p. 89–91°.

Lithium Aluminum Hydride Reduction of 2-Cyano-*m*-xylene.—To a stirred suspension of 11.4 g. (0.3 mole) of lithium aluminum hydride in 30 ml. of anhydrous ether was added dropwise a solution of 26.2 g. (0.2 mole) of 2-cyano-*m*-xylene in 350 ml. of anhydrous ether. After 25 ml. of the nitrite solution was added, the reaction was heated until it was self-sustaining, and the addition was completed with occasional heating when necessary. The mixture was refluxed 30 minutes and, after cooling in an ice-bath, the excess lithium aluminum hydride was decomposed by the dropwise addition of 50 ml. of water. This was followed by 500 ml. of 20% sodium potassium tartrate solution and the aqueous emulsion was separated from the ether solution and re-extracted with ether. The ether layers were combined, washed with water, dried over anhydrous magnesium sulfate and the ether removed by distillation. The residue was distilled twice to yield 22.7 g. (84%) of 2,6-dimethylbenzylamine, b.p. 96–98°.

2,6-Dimethylbenzyltrimethylammonium Iodide.—To a stirred mixture of 72.6 g. (0.51 mole) of methyl iodide and 200 ml. of water was added 12.0 g. (0.3 mole) of sodium hydroxide pellets. A solution of 19.0 g. (0.14 mole) of 2,6-dimethylbenzylamine in 20 ml. of ethanol was added at a rate to maintain the temperature at approximately 50°. After stirring 2 hr., the mixture was cooled in ice and the precipitated solid was collected by filtration and dried. The dried solid was dissolved in 20 ml. of boiling methanol and the crystalline product which separated after addition of an equal volume of absolute ether was collected by filtration; yield 32.2 g. (75%), m.p. 197–201°. A sample for analysis was recrystallized from absolute methanol and the product partially decomposed at 194°, finally melting at 199–204.

Anal. Calcd. for $C_{12}H_{20}NI$: C, 47.2; H, 6.61; N, 4.59; I, 46.6. Found: C, 47.1; H, 6.27; N, 4.66; I, 42.4.

Preparation of Methyl-substituted Diethyl Benzylacetamidomalonates. Diethyl 4-Methylbenzylacetamidomalonate (IIIa) (Method A).—To a stirred solution of 65.0 g. (0.35 mole) of *p*-xylyl bromide in 50 ml. of dry redistilled toluene was added 8.4 g. (0.35 mole) of sodium hydride, followed by 72.0 g. (0.33 mole) of diethyl acetamidomalonate. The suspension was stirred and refluxed under anhydrous conditions for 8 hr., at which time the evolution of hydrogen, as indicated by a bubble counter, had almost ceased. The unreacted sodium hydride was decomposed by the addition of a few ml. of absolute ethanol, followed by a few ml. of 50% aqueous ethanol and then sufficient water to bring the volume of the aqueous layer to approximately 200 ml. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layer and ether extracts were washed thoroughly with water, dried over anhydrous sodium sulfate and filtered. Concentration of the filtrate to approximately 100 ml. and cooling in an ice-bath caused a crystalline product to separate. Two crops yielded 78.0 g. (74%), m.p. 108–110°.

Diethyl 3,4-Dimethylbenzylacetamidomalonate (VIIIa) (Method B).—To a solution of 1.3 g. (0.057 g. atom) of sodium in 70 ml. of dry absolute ethanol[15] was added 12.4 g. (0.057 mole) of diethyl acetamidomalonate, followed by 7.9 g. (0.051 mole) of crude 3,4-dimethylbenzyl chloride. The mixture was refluxed for 3 hr. under anhydrous conditions and filtered. The addition of 200 ml. of water caused the separation of an oil which crystallized upon cooling. The product was collected by filtration; yield 9.0 g. (53%), m.p. 106–109°.

Diethyl 2,6-Dimethylbenzylacetamidomalonate (VIIa) (Method C).—To a solution of 1.04 g. (0.045 g. atom) of sodium in 150 ml. of dry absolute ethanol was added 10.9 g. (0.050 mole) of diethyl acetamidomalonate, followed by 375 ml. of dry xylene. The mixture was distilled with stirring until the temperature reached 135° and approximately 200 ml. of distillate was collected. Crystalline sodium diethyl acetamidomalonate separated at this point.[16] The condenser was arranged for reflux and 13.6 g. (0.045 mole) of 2,6-dimethylbenzyltrimethylammonium iodide was added. The suspension was stirred and refluxed for 20 hr. and filtered. The xylene from the filtrate was removed under vacuum on the steam-bath. The last traces of the solvent were removed by adding 20 ml. of water and distilling to dryness. The residue was dissolved in 200 ml. of 95% ethanol, treated with charcoal and Celite and filtered. An equal volume of water was added to the filtrate, and cooling and scratching produced a crystalline solid which was collected by filtration; yield 13.2 g. (88%), m.p. 98–99°.

Hydrolysis and Decarboxylation of Methyl-substituted Diethyl Benzylacetamidomalonates. 3,5-Dimethylphenylalanine (IX).—A suspension of 33.5 g. (0.1 mole) of IXa in 400 ml. of 25% hydrochloric acid was refluxed for 8 hr., and the solid which separated was collected by filtration and extracted with 125 ml. of boiling water. The insoluble material from the extraction was separated by filtration to give 6.5 g. of unhydrolyzed starting material. The filtrate was concentrated to 100 ml., cooled in an ice-bath and the crystals collected; yield 18.5 g. (99%) based on recovery of 6.5 g. starting material. A solution of 5.0 g. (0.022 mole) of this amino acid hydrochloride in 200 ml. of hot water was neutralized to *p*H 7.0 with concentrated ammonium hydroxide, and the precipitate which formed upon cooling in an ice-bath was collected by filtration; yield 3.9 g. (92%), m.p. 229–231° dec.

2,3-Dimethylphenylalanine (IV).—A suspension of 4.0 g. (0.012 mole) of IVa in 40 ml. of concentrated hydrochloric acid was refluxed for 6 hr. and filtered. The filtrate was cooled in an ice-bath and the crystals were collected by filtration; yield 2.2 g. (80%), m.p. 234–235° dec. A solution of 1.0 g. (0.0043 mole) of the amino acid hydrochloride

(14) The bulk of the material appeared to decompose and polymerize during the distillation to give a fluorescent, viscous oil. In subsequent preparations, the crude 3,4-dimethylbenzyl chloride was used without distillation in the condensation with diethylacetamidomalonate with satisfactory results.

(15) H. Lund and J. Bjerrum, *Ber.*, **64**, 761 (1946).

(16) If the diethyl acetamidomalonate is not pure or if anhydrous conditions are not used, a brown viscous oil sometimes separates and this results in a poor yield. When this situation is encountered, we have found it advantageous to recrystallize the diethyl acetamidomalonate from toluene.

in 50 ml. of hot water was neutralized to *p*H 7 with concentrated ammonium hydroxide. The solution was concentrated under vacuum on the steam-bath until a precipitate began to form. The mixture was cooled in an ice-bath and the precipitate collected. Two crops yielded 0.84 g. (100%), m.p. 237–242° dec.

2-Methylphenylalanine (II).—A suspension of 33.5 g. (0.11 mole) of IIa in 400 ml. of 20% hydrobromic acid was refluxed for 6 hr., and the solution was concentrated under vacuum on the steam-bath until a solid began to form. The mixture was cooled in an ice-bath and the crystals collected by filtration; yield 21.0 g. (78%). A solution of 18.0 g. (0.069 mole) of the amino acid hydrobromide in 200 ml. of water was added to an aqueous slurry of 70.0 g. of Amberlite IR-4B ion-exchange resin (basic form). The mixture was stirred and filtered and the resin washed with an additional 100 ml. of water. The combined filtrates were concentrated under vacuum until a precipitate formed. The solid which separated upon cooling in an ice-bath was collected. Two crops yielded 10.5 g. (85%), m.p. 240–243° dec.

KANKAKEE, ILLINOIS

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, THE ARMOUR LABORATORIES]

Analogs of Phenylalanine Containing Sulfur

BY ROBERT L. COLESCOTT, ROSS R. HERR AND JOSEPH P. DAILEY

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Analogs of phenylalanine containing a sulfur group in the *para* position have been synthesized and tested as growth inhibitors of *Escherichia coli*. The mercapto, symmetrical disulfide, methylmercapto, ethylmercapto, methylsulfinyl, methyl sulfonyl and sulfamyl analogs were prepared by hydrolysis and decarboxylation of the corresponding diethyl acylamidomalonates.

The substitution of various groups on the benzene ring of phenylalanine has often resulted in compounds that are competitive antagonists of phenylalanine or tyrosine, the *para* substituted compounds being the most active. Some of these *para* substituted analogs of phenylalanine that have been previously reported include the nitro,[1] amino,[1] fluoro,[2] chloro,[1] methyl,[3] dimethylamino,[4] mercapto,[5,6] sulfamyl[7,8] and the symmetrical disulfide.[5,6] Because of the potential usefulness of amino acid antagonists in chemotherapy, we have prepared a group of compounds related to phenylalanine wherein a sulfur containing group is substituted in the *para* position of this amino acid. In these compounds, variations in the oxidation state of the sulfur results in groups having a range of electronic effects, from the electron-donating mercapto group to the electron-withdrawing sulfonamide. In this way we hoped to obtain information about the relationship between electronic character of the substituent and antagonism or utilization of the amino acid. We have prepared the mercapto, disulfide, methylmercapto, ethylmercapto, methylsulfinyl, methylsulfonyl and sulfamyl substituted phenylalanines.

The general method of preparation of these amino acids was by way of the corresponding diethyl acetamidomalonate (Table I).

The amino acid was then prepared by hydrolysis and decarboxylation (Table II).

The preparation of *p*-mercaptophenylalanine was first reported by Johnson and Brautlecht[5] and later by Elliott and Harington.[6] A more direct route and one which resulted in higher yield was found in the reaction of potassium ethyl xanthate with diethyl *p*-diazobenzylacetamidomalonate. The diazo-compound was prepared readily from the corresponding amine and this, in turn, was obtained by hydrogenation of diethyl *p*-nitrobenzylacetamidomalonate.[1] The hydrolysis of the xanthate, the hydrolysis of the amide and ester groups, as well as the decarboxylation, were accomplished in one step to give the amino acid hydrochloride in 86% yield. Oxidation of the *p*-mercaptophenylalanine to the corresponding disulfide was carried out according to the procedure of Elliott and Harington using iodine in potassium iodide solution.

Chloromethylation of thioanisole or thioanethole with chloromethyl ether yielded the *para* substituted benzyl chloride. The thioanethole was prepared by the reaction of diethyl sulfate with thiophenol. Condensation of these benzyl halides with diethyl acetamidomalonate resulted in the production of the substituted acetamidomalonates which were then hydrolyzed and decarboxylated to yield *p*-methylmercaptophenylalanine and *p*-ethylmercaptophenylalanine.

Controlled oxidation of the diethyl *p*-methylmercaptobenzylacetamidomalonate with hydrogen peroxide resulted in the sulfinyl substituted malonate and hydrolysis of this yielded *p*-methylsulfinylphenylalanine. Oxidation with an excess of hydrogen peroxide gave the sulfone which was hydrolyzed to yield the corresponding sulfonyl amino acid.

Two methods were employed successfully in the preparation of *p*-sulfamylphenylalanine.[7] Chlorosulfonation of diethyl benzylacetamidomalonate followed by reaction with ethereal ammonia and hydrolysis and decarboxylation resulted in the sulfonamide in 16% yield. The alternate procedure utilized side chain bromination of *p*-toluene-

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