[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF KANSAS SCHOOL OF PHARMACY]

Synthesis of Phenylalanine Analogs as Antimetabolites

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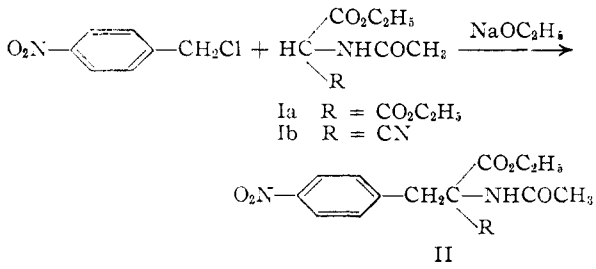
Many studies are currently underway in various laboratories and much has already been done[2] toward finding antimetabolites which might also be useful chemotherapeutic agents. A number of compounds developed through this approach have exhibited antimetabolic properties, but because of various shortcomings, such as low absorption, low activity or high toxicity, these agents have failed to become established in medical practice. Although a number of widely used medicinals are known to be metabolite antagonists, the fact was discovered after their establishment as effective drugs. Despite the past disappointing results from applications of the antimetabolite concept, it remains as one of the few logical and appealing approaches to the problem of finding more useful chemotherapeutic agents, and it can be said that the proper compounds may not have yet been chosen for synthesis.

The observation that antimalarial agents and certain insecticides hold large space-occupying groups, such as the chlorophenyl radical, which presumably exert desired toxic effects, and another portion of the molecule as a solubilizing group which allows absorption at the proper site, suggested the preparation of a variety of unnatural amino acids which would contain large substituents and leave free the functional groups either for solubilizing purposes or peptide synthesis within the organism.[3] Interest in such compounds particularly as possible antiviral agents seems justified by the knowledge that viruses are composed largely of protein materials or amino acids.[4] Phenylalanine analogs were chosen to compose this first described section of proposed chemical studies, because phenylalanine is an essential amino acid not only to man but to other forms of life. Further, the preparation of the compounds in good yield appeared to be feasible because of the development of useful synthetic procedures[5] and the availability of intermediate substituted benzyl halides. As shown by Table II, a variety of substituents was used in order to afford a study of the relative magnitudes of antimetabolic effect by both large and small groups.[6]

General procedure A, essentially that of others,[5] was employed in the preparation of most of the esters of Table I. The method is illustrated by the preparation of II. The malonates were hydrolyzed with concentrated hydrochloric acid and the

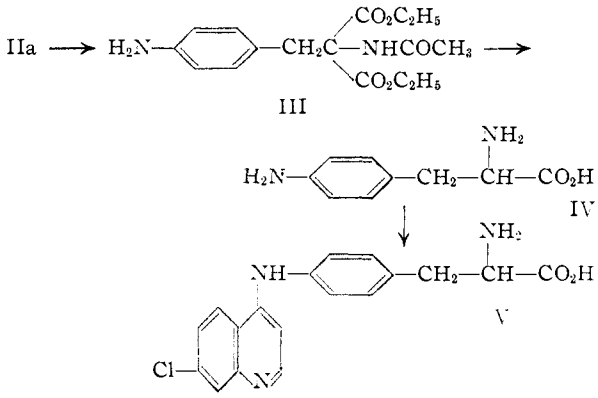


Ia R = $CO_2C_2H_5$
Ib R = CN

II

cyanoacetates with hydrobromic acid to yield the amino acids of Table II. While the twelve esters of Table I are new compounds, a number of the amino acids of Table II had been previously prepared by other methods.[7] However, apparently they had not been made for antimetabolite studies.

The presence of the 7-chloro-4-quinolylamino grouping in valuable antimalarial agents, such as Chloroquine and Camoquin, suggested β-[DL-*p*-(7-chloro-4-quinolylamino)]-phenylalanine (V) as a compound of possible biological interest. V was prepared in good yield by condensation of 4,7-di-



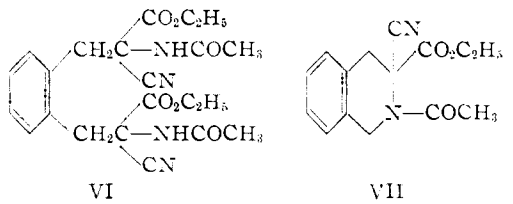
III

IV

V

chloroquinoline with IV which had been obtained by hydrolysis of III which in turn had been obtained by catalytic reduction of IIa.

In the condensation of *o*-xylylene bromide with Ib, not only was the expected ester VI prepared in 30% yield, but ethyl 2-acetyl-3-cyano-1,2,3,4-tetrahydro-3-isoquinoline carboxylate (VII) was isolated in 43% yield.



VI VII

(1) Fellow of the American Foundation for Pharmaceutical Education (Procter and Gamble grant, 1948-1950).

(2) Roblin, *Chem. Revs.*, **38**, 255 (1946).

(3) Many of the unnatural amino acids thus far prepared have contained nuclei that are isosteric with those of the natural relatives, while others have been characterized by a substitution of one relatively small group by another. For example, see Dittmer, *et al.*, *J. Biol. Chem.*, **164**, 761 (1946).

(4) Stanley, *Chem. Eng. News*, **51**, 3786 (1946); Elvehjem, *J. Am. Med. Assoc.*, **136**, 915 (1948).

(5) Snyder, Shekleton and Lewis, THIS JOURNAL, **67**, 310 (1945); Albertson and Archer, *ibid.*, **67**, 308 (1945); Albertson and Tullar, *ibid.*, **67**, 502 (1945).

(6) Recent papers on phenylalanine analogs: Nevenzel, Shelberg and Neimann, *ibid.*, **71**, 3024 (1949); Elliot, Fuller and Harington, *J. Chem. Soc.*, 85 (1948).

(7) It is well known that the decomposition points of amino acids depend upon the method of determination. The values reported in Table II were obtained by using a capillary tube in an electrically heated bath of silicone fluid. In general, the bath was preheated to 200° and then heated rapidly until the decomposition point was reached. Any disagreement with prior reports may be attributed to differences in the mechanics of the determinations. In cases where values vary significantly, elementary analyses were obtained.

TABLE I

INTERMEDIATE ACETAMIDOMALONATES AND ACETAMIDOCYANOACETATES

	Name	Proced.[i]	Yield,[b] %	M. p.,[c] °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
	Diethyl x-benzylacetamidomalonates								
1	*o*-Chloro	A	81	94–95[d]	$C_{16}H_{20}ClNO_5$	56.22	56.44	5.90	5.62
2	*p*-Chloro	A	84	143–144	$C_{16}H_{20}ClNO_5$	56.22	55.82	5.90	5.55
3	3,4-Dichloro	A	89	134–136	$C_{16}H_{19}Cl_2NO_5$	51.08	51.30	5.09	5.05
4	2-Ethoxy-5-nitro	A	82	156–158	$C_{18}H_{24}N_2O_8$	54.54	54.75	6.10	6.10
5	2-Hydroxy-5-nitro	[a]	20	184–198 dec.[e]	$C_{16}H_{20}N_2O_8$	52.17	52.32	5.47	5.84
6	*p*-Nitro (IIa)	A	88	193–194	$C_{16}H_{20}N_2O_7$	54.53	54.78	5.72	5.91
7	*p*-Amino (III)	[a]	97	216–222 dec.	$C_{16}H_{22}N_2O_5 \cdot HCl$[h]				
	Ethyl α-x-aralkyl-α-acetamido-α-cyanoacetates								
8	*p*-Nitrobenzyl (IIb)	A	92	185–187	$C_{14}H_{15}N_3O_5$	55.08	55.54	4.95	4.87
9	*p*-Aminobenzyl	[a]	57	141–142	$C_{14}H_{17}N_3O_3$	61.07	61.48	6.23	6.22
10	Benzohydryl	A	35	188–189[f]	$C_{20}H_{20}N_2O_3$	71.41	71.97	5.99	6.08
11	Ethyl α,α′-*o*-xylylene-bis-(α-acetamido-α-cyanoacetate) (VI)	[a]	30	224–228 dec.[g]	$C_{22}H_{26}N_4O_6$	59.72	59.82	5.92	5.86
12	Ethyl 2-acetyl-3-cyano-1,2,3,4-tetrahydro-3-isoquinoline carboxylate (VII)	[a]	43	143–145	$C_{17}H_{22}N_2O_4$	66.16	66.20	5.92	5.80

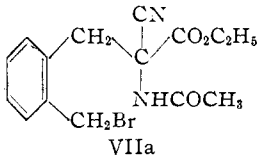
[a] See experimental part. [b] Yields are based upon weight of crude product since in general no purification was required before hydrolysis. [c] Melting point of the analytical sample; when m. p. of crude sample is more than two degrees lower, it will be indicated in footnotes. The esters in this table were recrystallized from either absolute alcohol or diluted alcohol. [d] Crude 77–85°. [e] Melts at 209–211° dec. when bath preheated to 200°. [f] Crude 170–178°. [g] Crude 208–210°. [h] *Anal.* Calcd.: Cl, 9.88. Found: Cl, 9.78. [i] Source of intermediate substituted benzyl halides given by compound numbers: 1, 6, 8, 11 and 12 from Eastman Kodak Co.; 2 and 3 gratis from Heyden Chemical Corporation, Garfield, New Jersey; 4 from Burckhalter *et al.*, THIS JOURNAL, **70**, 1372 (1948); 5 from *Org. Syntheses*, **20**, 59 (1940); 10 from Halogen Chemicals, Inc., Columbia, S. C.

TABLE II

DL-β-(SUBSTITUTED-PHENYL)-ALANINES

	Name	Proced.	Yield, %	M. p. (dec.),[a] °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	*o*-Chloro	B	88	226–228[b]	$C_9H_{10}ClNO_2$	54.14	53.95	5.05	5.03
2	*p*-Chloro	B	68	236–241[c]					
3	3,4-Dichloro	B	>90	229–234	$C_9H_9Cl_2NO_2$	46.18	45.86	3.88	4.02
4	*m*-Methyl	B[d]	69	242–246[e]					
5	2-Ethoxy-5-nitro	B	84	227–230	$C_{11}H_{14}N_2O_5 \cdot H_2O$	48.52	48.56	5.92	6.03
6	2-Hydroxy-5-nitro	B	95	252–257	$C_9H_{10}N_2O_5$	47.79	47.74	4.46	4.17
7	*p*-Nitro	B	78	222–240[f]	$C_9H_{10}N_2O_4$	51.43	51.17	4.80	4.89
8	*p*-Amino (IV)	B	81	235–242[g]	$C_9H_{12}N_2O_2 \cdot 1^1/_2H_2O$[h]				
9	*p*-(7-Chloro-4-quinolylamino) (V)		79	236–237	$C_{18}H_{16}ClN_3O_2 \cdot HCl \cdot 1^1/_2H_2O$	53.34	53.43	4.97	4.62
10	β,β-Diphenylalanine	B	70	234–240[i]					
11	β,β′-(*o*-Phenylene)-dialanine	B	94	>270	$C_{12}H_{16}N_2O_4 \cdot 3H_2O$	47.05	47.30	7.24	7.22
12	1,2,3,4-Tetrahydro-3-isoquinoline-carboxylic acid	B	79	324–325[j]	$C_{10}H_{11}NO_2$	67.78	67.71	6.26	6.20

[a] The bath was generally preheated to 200° and then the temperature raised rather rapidly. Most of the products were recrystallized from water or diluted alcohol. [b] Using a different synthetic method, Henze, Whitney and Eppright, THIS JOURNAL, **62**, 565 (1940), found 260–261°. [c] By a different procedure, Friedmann and Maase, *C. A.*, **4**, 3094 (1910), found 243–244°. [d] The intermediate acetamidomalonate could not be easily isolated as a solid. The sodium chloride was removed, as in procedure A, and the solution reduced in volume to an oil *in vacuo*. Using the oil, procedure B was applied. [e] Boehm, *Z. physiol. Chem.*, **89**, 101 (1914), found 245°. [f] Erlenmeyer and Lipp, *Ann.*, **219**, 219 (1883), found 240–245° by a different preparative method. [g] Erlenmeyer and Lipp found 245–250°. [h] *Anal.* Calcd.: N, 13.50. Found: N, 13.53. [i] By a different method, Harington and McCartney, *J. Chem. Soc.*, 892 (1929), found 236°. [j] Bath preheated to 280°. When not preheated, m. p. is 306–311°. Pictet and Spengler, *Ber.*, **44**, 2031 (1911), found 311°.



VIIa

Apparently, VII was formed *via* intramolecular alkylation of the unisolated intermediate VIIa.

Theoretically, VI should exist as a DL pair and a *meso* isomer. In one fortuitous experiment there was a yield of 30% of VI (m. p. 208–210° dec.) and 70% of VII. Two recrystallizations brought the melting point of VI to a constant 224–228° dec. which is suggestive of the presence of a single pure substance, either the DL pair or *meso* isomer. However, the configuration of pure VI and its hydrolytic product, β,β-(*o*-phenylene)-dialanine, has not yet been determined.

1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid, made by the hydrolysis of VII, had previously been prepared by chloromethylation of phenylalanine, and the same decomposition points were observed.[8] The structures of the acid and the intermediate ester VII are considered to have been confirmed by the method of synthesis and the elementary analyses,

(8) Pictet and Spengler, *Ber.*, **44**, 2031 (1911).

Biological Results.—Of the Table II compounds tested as inhibitors of the growth of *E. coli* by Dr. R. C. Mills and Mr. J. H. Fellman, of the University of Kansas, DL-β-(*p*-aminophenyl)-alanine was the most active. At 0.5 γ/ml. of medium there was 50% inhibition of growth. Compounds 2, 9 and 10 were similarly effective at the much higher level of 1000 γ/ml., while compounds 6 and 7 were inactive at that level. The competitive nature of the inhibition was demonstrated with either tyrosine or phenylalanine. Greater details will be published elsewhere.

Drs. Arthur Furst and Harold A. Harper of the University of San Francisco will also report elsewhere the results of their studies with this series of amino acids.

Acknowledgment.—The authors are indebted to the American Foundation for Pharmaceutical Education and the Procter and Gamble Company for a fellowship grant to one of us (V. C. S.).

Experimental[9]

Procedure A.—Seven of the esters of Table I were obtained by the following general method. To an absolute alcohol solution of the sodium salt of ethyl acetamidomalonate (Ia) or of the cyanoacetate (Ib) made from an equivalent amount of freshly prepared sodium ethoxide, there was added an equimolar portion of the appropriate benzyl halide. The reaction was carried out in a three-necked flask equipped with a stirrer and condenser, protected from atmospheric moisture by a drying tube. The reaction time varied from two hours, without external heating in the case of the *p*-nitro compound, to six hours at reflux temperature with less reactive halides. The precipitated sodium halide was removed by filtering the hot solution and the product isolated by concentration of the filtrate under water-pump vacuum or merely by cooling.

Procedure B.—The amino acids of Table II were prepared by acid hydrolysis of the appropriate ester. For 0.1 mole of an acetamidomalonate, 150 ml. of 40% hydrobromic acid was utilized, and the reaction mixture was refluxed for periods ranging from six hours to overnight. However, the reaction is probably complete within the shorter period. The cyanoacetates were hydrolyzed in concentrated hydrochloric acid—using the same ratio of acid to ester—by refluxing four to six hours. If an insoluble salt formed, it was separated by filtration after the heating period and dissolved in a slight excess of ammonia solution. Then the *p*H was adjusted to 6 by use of glacial acetic acid. In other instances the hot solution was filtered, cooled and brought to a *p*H of 6 by means of ammonia solution. The solution was then cooled in the refrigerator and the solid amino acid which formed was removed and washed with ice-water. In some cases concentration of the filtrate *in vacuo* gave a second crop of product. The amino acids were purified by recrystallization from water or aqueous alcohol.

Diethyl 2-Hydroxy-5-nitrobenzylacetamidomalonate.—The procedure A was used except that two equivalents of sodium were required. At the end of the reaction period, an excess of hydrogen chloride was passed into the solution, the sodium chloride formed was removed by filtration and the filtrate was concentrated under reduced pressure. The resulting solid was recrystallized from alcohol. The melting point was not changed by repeated recrystallizations.

Diethyl *p*-Aminobenzylacetamidomalonate Hydrochloride (III).—A suspension of 35 g. (0.1 mole) of diethyl *p*-nitrobenzylacetamidomalonate (IIa) in 550 ml. of absolute alcohol was subjected to reduction using Adams catalyst at three atmospheres of hydrogen. It was found necessary to heat the reaction bottle (infrared lamp), probably to keep sufficient IIa in solution, in order to obtain satisfactory results. After three hours, the catalyst was removed, and the solution saturated with dry hydrogen chloride. After cooling, the solid precipitate was combined with that realized from evaporation of the filtrate, total yield 34.5 g. Recrystallization from absolute alcohol did not change the melting point.

Ethyl α-(*p*-Aminobenzyl)-α-acetamido-α-cyanoacetate.—By the procedure for the preparation of III, 116 g. (0.39 mole) of ethyl α-(*p*-nitrobenzyl)-α-acetamido-α-cyanoacetate (IIb) was reduced during a period of 48 hours to 61 g. (57%) of desired free base; m. p. 137–140°. Isopropyl alcohol proved to be the best recrystallizing solvent.

DL-β-[*p*-(7-Chloro-4-quinolylamino)-phenyl]-alanine Monohydrochloride (V).—A mixture of 3.5 g. (0.018 mole) of 4,7-dichloroquinoline and 3.5 g. (0.017 mole) of IV in alcohol containing one ml. of alcoholic hydrogen chloride was warmed for a few minutes and allowed to stand for twelve hours. A yellow solid product V separated; yield 5.5 g., m. p. 236° dec. No satisfactory means of recrystallization was found for the bulk of the material, but an analytical sample was obtained by using alcohol in a Soxhlet extractor.

Condensation of Ethyl α-Acetamido-α-cyanoacetate (Ib) with Xylylene Bromide.—To the sodium salt of 34 g. (0.2 mole) of the cyanoacetate (Ib) in 150 ml. of anhydrous alcohol solution, 26.4 g. (0.1 mole) of xylylene bromide was added with stirring during seven hours at reflux temperature. The solid, after filtration from the hot mixture, was treated with water for removal of the sodium bromide. The insoluble portion upon recrystallization from dilute alcohol yielded 8 g. of VI, m. p. 218–226° dec. The alcohol filtrate from the original hot mixture upon cooling gave 34 g. of mixed solids, m. p. 140–180°. Treatment with boiling chloroform, gave 19 g. of crude VI (m. p. 210–212° dec.) or a total yield of 27 g. (30%). Reduction in the volume of the filtrate and the addition of ether gave 23 g. (43%) of VII, m. p. 140–145°. Both products were conveniently recrystallized from dilute alcohol.

The foregoing description indicating a total yield of 72% is representative of several repetitions of the same experiment. However, the first run involved very fortuitous separations, which could not be repeated. The sodium bromide separated in quantitative yield. A reduction in volume of the alcohol filtrate gave VI (m. p. 208–210°) in about 30% yield, and the addition of ether to the second alcohol filtrate gave VII (m. p. 138–142°) in about 70% yield.

Summary

A group of twelve phenylalanine analogs was prepared as possible metabolite antagonists.

Ethyl 2-acetyl-3-cyano-1,2,3,4-tetrahydro-3-isoquinoline carboxylate (VII) was unexpectedly obtained as the principal product in the condensation of sodium α-acetamido-α-cyanoacetic ester and xylylene bromide.

DL-β-(*p*-Aminophenyl)-alanine was found to be an inhibitor of the growth of *E. coli* in competition with either tyrosine or phenylalanine.

(9) Microanalyses by Mr. C. W. Beazley, Skokie, Ill.

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