conjugated systems by the methylene group, the benzyl group of Xb binds directly to the enzyme in some fashion such as a charge-transfer complex[6-8] or less likely by hydrophobic binding.[14,15]

Whether the 6-phenyl or 6-benzyl groups of X aand Xb bind in the same fashion still cannot be resolved at this point; that is, the 6-phenyl might influence pyrimidine binding and the 6-benzyl might bind directly. Nevertheless, the positive results remain that the 6-phenyl, 6-benzyl, or 6-furyl groups of Xa, Xb, and Xe increase binding compared to a 6-methyl group, and an electron-withdrawing nitro group as in Xc decreases binding.

It was previously observed that replacement of the 4-hydroxyl group of 2-amino-5-(3-anilinopropyl)-4-pyrimidinol (III) by amino (XXIII) gave a 2600 enhancement in binding[8] and by 4-mercapto (XXIV) a 14-fold enhancement.[7] In another paper of this series it was noted that replacement of the anilinopropyl group of III by a 4-phenylbutyl (XXII) also led to an enhancement of binding.[7] In Table II, a comparison of the effects of these three substitutions on binding of the 6-phenyl and 6-benzyl groups of Xa and Xb is made.

The 35-fold enhancement of 6-phenyl (Xa) over 6-methyl (Xb) in group A (Table II) with an anilinopropyl group is maintained in group B when the phenylbutyl side chain is present (XXII *vs.* XIXa). In contrast this increment is almost completely lost in the 6-benzyl series (XIXb *vs.* XXII); thus the phenylbutyl side chain causes a decrease in the binding of the 6-benzyl group or *vice versa*, or both.

A further enhancement in binding by the 6-phenyl or 6-benzyl moieties in the 4-amino series (groups C and D) is not observed; of interest is the fact that 6-benzyl group causes a decrease in binding compared to the 6-methyl group with the anilinopropyl side chain (group C), but had no detrimental effect with the phenylbutyl series (group D). Similarly, a further enhancement in binding by the 6-phenyl group in the 4-mercapto series (group E) was not observed; in fact, the 6-phenyl (XVIIa) was about one-half as effective as a 6-methyl (XXIV). This lack of additivity with the 6-phenyl moiety in groups C, D, and E (Table II) could be attributed to the fact that the 4-amino and 4-mercapto groups on the pyrimidine already have the most favorable tautomeric form and can no longer be influenced by the phenyl ring as in group A; however, such an explanation for the 6-benzyl moiety is untenable. One possible explanation is compatable with both the 6-benzyl and 6-phenyl systems; when the 4-amino and 4-mercapto groups are bound to folic reductase, a conformational change results in the enzyme which no longer allows binding of these aryl moieties. If this explanation is correct, it should be possible to construct irreversible inhibitors that will detect this enzymic conformational change by utilization of the bridge principle of specificity[16]; currently a search for such types of irreversible inhibitors is underway.

Furthermore, even though the three ways of increasing the binding of III are not additive, placement of a phenyl or benzyl group at the 6-position of III does not decrease binding (except in the case of XVIIIb which is not a serious loss); thus it should be feasible to synthesize a variety of potential irreversible inhibitors with covalent bond forming groups built off the 6-position in molecules related to XVIII, XXI, or XXIII.

(14) R. A. Wallace, A. N. Kurtz, and C. Niemann, *Biochemistry*, **2**, 824 (1963).

(15) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

(16) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964).

Amino Acid Analogs of Tryptamine Antagonists of Serotonin

FREDERICK LEONARD[1] AND WALTER TSCHANNEN

Department of Medicinal Chemistry, Geigy Research Laboratories, Ardsley, New York

Received November 20, 1964

Amino acid analogs of two tryptamine antagonists of serotonin were prepared for biological evaluation. The 1-benzyltryptophans prepared in this study, like 1-benzyl-5-methoxy-2-methyltryptamine (BAS), produce sedation in mice while a nonbenzylated tryptophan caused stimulation.

Serotonin[2] (5-hydroxytryptamine) and the serotonin antagonist, 1-benzyl-5-methoxy-2-methyltryptamine[3] (BAS), do not readily cross the blood–brain barrier. The biological precursor of serotonin, 5-hydroxytryptophan, however, is transported into the brain where it is decarboxylated to serotonin.[2] It was conjectured, therefore, that amino acid analogs of BAS might possess interesting biological properties. Several tryptophan derivatives (**1a–1c**) were consequently prepared in these laboratories to explore this possibility.[4]

Two methods were considered for the preparation of the desired amino acids, the Warner–Moe[5] tryptophan

(1) Psychopharmacology Service Center, National Institute of Mental Health, Bethesda, Md. 20014.

(2) S. Udenfriend, H. Weissbach, and D. F. Bogdanski, *J. Biol. Chem.*, **224**, 803 (1957).

(3) (a) D. W. Wooley and E. N. Shaw, *Science*, **124**, 34 (1956); (b) D. W. Wooley, E. Van Winkle, and E. N. Shaw, *Proc. Natl. Acad. Sci. U. S.*, **43**, 128 (1957).

(4) G. Domschke and G. Muller, *J. prakt. Chem.*, [4] **21**, 85 (1963), described the preparation, by a different synthesis than what we employed, of one of the tryptophans (**1d**) we had intended to synthesize.

(5) D. T. Warner and O. A. Moe, *J. Am. Chem. Soc.*, **70**, 2764 (1948), prepared the key tryptophan intermediate, diethyl acetamido(3-indolylmethyl)malonate (b) by Fisher cyclization of the phenylhydrazone of diethyl acetamido(β-formylethyl)malonate (a) and converted it to DL-tryptophan by previously described procedures.

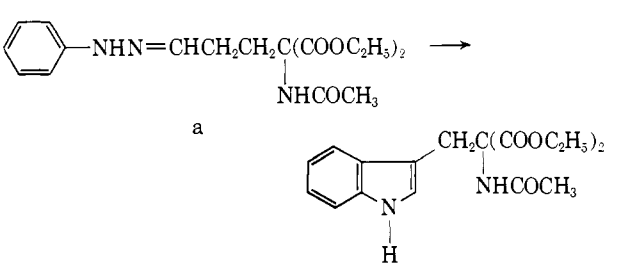
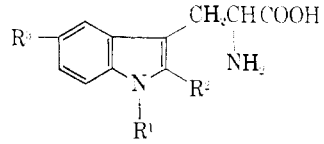


TABLE I
GROSS PHARMACOLOGICAL OBSERVATIONS IN MICE[a]

Compd.[b]	LD_{50}[c]	Symptomatology 50 mg./kg. i.p.	Symptomatology 100 mg./kg. i.p.
1a		Normal	At 15 min. animals very active; at 40 min. ataxia; at 1 hr. active, piloerection; at 4 hr. same as at 1 hr.; recovery after 7.5 hr.
1b	135	At 5 min. normal; at 30 min. quiet; at 2 hr. quiet, eyes open, move freely; at 7 hr. normally active.	At 5 min. tremors; at 30 min. sedation; at 2 hr. deep sedation, eyes closed, righting reflex (RR) reduced; at 3.5 hr. RR barely present, deep sedation; at 7 hr., deep sedation, RR present, slight piloerection; recovery in 2 mice at 24 hr; 3 mice still sluggish after 24 hr.
1c	130	At 5 min. excitement; at 30 min. quiet; at 3.5 hr. quiet but move when stimulated; at 8 hr. quiet; at 24 hr. normal	At 5 min. partial hind limb paralysis; at 15 min. quiet; at 2 hr. complete sedation but can be aroused, RR present; at 8 hr. still well sedated, RR present; at 24 hr. normal.

[a] Five animals per group. [b] Compounds were administered as 1% solutions in propylene glycol. [c] 24 hr.

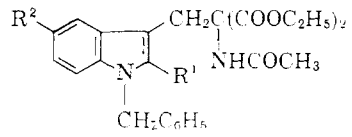


1a, R^1 = H; R^2 = CH_3; R^3 = CH_3O
b, R^1 = $CH_2C_6H_5$; R^2 = CH_3; R^3 = H
c, R^1 = $CH_2C_6H_5$; R^2 = H; R^3 = CH_3O
d, R^1 = $CH_2C_6H_5$; R^2 = CH_3; R^3 = CH_3O

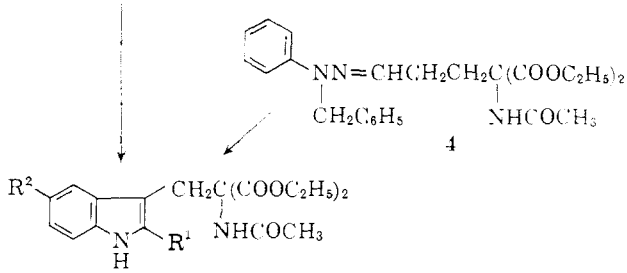
synthesis and N-benzylation of amino acid intermediates. Cornforth, *et al.*,[6] employed N-benzyl-N-phenylhydrazine instead of phenylhydrazine in the Warner-Moe tryptophan synthesis and obtained 1-benzyltryptophan. All of our attempts, however, to condense *p*-methoxyphenylhydrazine or N-benzyl-N-(*p*-methoxyphenyl)hydrazine with diethyl acetamido(β-formylethyl)malonate[5] or diethyl acetamido(3-oxobutyl)malonate gave oily products which decomposed when subjected to typical indole cyclization conditions.

In a synthesis of 1-benzyltryptophans from indole intermediates, the benzyl group may be introduced at various stages of the synthesis. We chose to study the benzylation of indoleacetamidomalonates (**2**) since two of the requisite intermediates were known compounds and the synthesis of the third intermediate, diethyl acetamido(5-methoxy-2-methyl-3-indolylmethyl)malonate (**2d**) from 5-methoxy-2-methylindole[7] appeared to offer no great difficulties.

The feasibility of benzylating indoleacetamidomalonates (**2**) was evaluated in benzylation experiments with the readily available diethyl acetamido(3-indolylmethyl)malonate[5] (**2a**); only sodamide-catalyzed benzylation[8] in liquid ammonia gave a satisfactory yield of a product (**3a**) that was identical with that obtained by Cornforth and co-workers[6] from the cyclization of the N-benzyl-N-phenylhydrazone (**4**) of diethyl acetamido(β-formylethyl)malonate. Benzylation products were later obtained in somewhat better yield from **2b** and **2c** than from **2a**. 1-Benzyltryptophans (**1b** and **c**) were obtained in the usual way from **3b** and **3c** while the available supply of **2d** was converted to the amino acid analog (**1a**) of 5-methoxy-2-methyltryptamine, another of the high-potency serotonin antagonists described by Shaw and Wooley.[9]



2a, R^1 = R^2 = H
b, R^1 = CH_3; R^2 = H
c, R^1 = H; R^2 = CH_3O
d, R^1 = CH_3; R^2 = CH_3O



3a, R^1 = R^2 = H
b, R^1 = CH_3; R^2 = H
c, R^1 = H; R^2 = CH_3O

Compounds **1b** and **1c** produced sedation in mice in doses comparable to that at which BAS demonstrated pharmacological activity while compound **1a** was found to be somewhat less potent. The pharmacological effects of these compounds at doses of 50 and 100 mg./kg. i.p. in mice are tabulated in Table I. It is noteworthy that **1a** which does not have a benzyl group at position 1 caused excitation while both 1-benzyltryptophans (**1b** and **1c**) produced sedation.

Wooley, *et al.*,[3b] found that the effects of BAS in mice are quite different after intraperitoneal and intracerebral administration and concluded, therefore, that BAS penetrated the brain poorly. BAS, for example, at a dose of 1 mg./mouse i.p. (*ca.* 50 mg./kg.) caused mice to be somewhat more quiet than normal controls, while 30 γ/mouse[10] injected into the left ventricle of the

(6) J. W. Cornforth, R. Cornforth, C. E. Dalgleish, and A. Neuberger, *Biochem. J.*, **48**, 591 (1957).
(7) N. Nenitzescu, *Bull. soc. chim. Romania*, **11**, 37 (1929).
(8) H. Pleininger, *Chem. Ber.*, **87**, 127 (1954).
(9) E. N. Shaw and D. W. Wooley, *J. Pharmacol. Exptl. Therap.*, **116**, 164 (1956).
(10) The quantity calculated by Wooley, *et al.*,[3b] which should reach the brain after intraperitoneal injection of 1 mg./mouse if the drug is uniformly distributed to all parts of the body.

brain caused excitation. The sedative effects of compounds **1b** and **1c** at a dose of 50 mg./kg. i.p. suggest that they either, like BAS, do not cross the blood–brain barrier or, if they do so, are not decarboxylated to BAS congeners. It would seem that **1a** which is closely related to normal tryptophan metabolites might have crossed the blood–brain barrier. It appears, at least, to have the best chance of compounds **1a–1c** of penetrating into the brain. At this juncture, analyses of brain tissue from animals fed these compounds are required to resolve the issues of their penetration into the brain and the form in which they act.

Experimental[11]

Diethyl Acetamido(3-oxobutyl)malonate.[12]—A solution of methyl vinyl ketone (16.2 ml., 0.2 mole) in 15 ml. of dry benzene was added dropwise with stirring to a slurry of 43.4 g. (0.2 mole) of diethyl acetamidomalonate and 0.2 g. of sodium methoxide in 65 ml. of dry benzene. The temperature of the reaction mixture slowly rose to 35° during 30 min. The mixture was stirred for 20 hr. at room temperature, cooled with ice water, and filtered. The crystalline product, after recrystallization first from a 1:1 mixture of benzene and hexane (yield 47.8 g., 83.2%, m.p. 86–87°) and then from ethanol, melted at 88–89°.

Anal. Calcd. for $C_{13}H_{21}NO_6$: C, 54.34; H, 7.37; N, 4.88. Found: C, 54.39; H, 7.56; N, 5.17.

Diethyl Acetamido(5-methoxy-2-methyl-3-indolylmethyl)-malonate (2d).—Diethyl acetamidomalonate (5.75 g., 0.027 mole) and 6.8 g. (0.026 mole) of 5-methoxy-2-methyl-3-piperidinomethylindole[13] were added stepwise to a cooled (*ca.* 50°) solution of sodium ethoxide prepared from 0.65 g. (0.026 g.-atom) of sodium and 75 ml. of absolute ethanol. The resulting mixture was warmed to 60–70° to dissolve the reagents, cooled to 30°, and treated with excess dimethyl sulfate (5 ml.) (cooling to 30–40°) during 0.5 hour.

The mixture was then stirred for 4 hr. at room temperature, stored overnight at room temperature, and poured into ice–water. The resulting mixture was stored in the refrigerator overnight. The crude indolemalonate was filtered off and washed with water. It was dissolved in ethanol and reprecipitated by pouring into ice–water (yield 8.4 g., 69.5%, m.p. 153–155°). A sample of the precipitate melted at 160° after recrystallization from isopropyl alcohol.

Anal. Calcd. for $C_{20}H_{26}N_2O_6$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.80; H, 6.58; N, 7.49.

DL-5-Methoxy-2-methyltryptophan (1a).—A suspension of 8.4 g. (0.0215 mole) of diethyl acetamido(5-methoxy-2-methyl-3-indolylmethyl)malonate in a solution of 5.3 g. of NaOH in 26 ml. of water and 52 ml. of ethanol was refluxed for 3 hr. and evaporated to dryness *in vacuo*. Water (50 ml.) was added to the residue and the resulting solution was filtered, extracted twice with an 8:2 mixture of chloroform and 1-butanol, and acidified with 14.7 ml. of concentrated HCl with ice cooling. The acidified mixture was covered with 10 ml. of 8:2 chloroform–1-butanol and was refrigerated for 3 days. The precipitated malonic acid was filtered off, washed with water, and dried; yield 5.6 g. (78%), m.p. 135–137°.

The malonic acid (5.6 g., 0.0167 mole) was decarboxylated by heating at 180–200° for 1.5 hr. Barium hydroxide solution [12.8 g. of $Ba(OH)_2$ in 42 ml. of water] was added to the decarboxylation residue, and the mixture was refluxed for 24 hr., cooled, and acidified with H_2SO_4. The hot mixture was filtered, and the precipitate was extracted first with hot water and then with ethanol. The combined aqueous solutions were evaporated to dryness, and the residue was recrystallized from the minimum volume of water. The crystalline product was filtered off, washed with cold alcohol, and dried; yield 1.53 g. (37%), m.p. 269–270°.

Anal. Calcd. for $C_{16}H_{18}N_2O_6$: C, 62.88; H, 6.50; N, 11.28. Found: C, 62.85; H, 6.63; N, 11.21.

The recrystallization filtrate and washings were combined with the alcoholic extract and evaporated to dryness. The residue was taken up in ammonia, and the solution was evaporated to dryness. The solid residue was recrystallized and gave an additional 0.75 g. of product, m.p. 269–270°.

Diethyl Acetamido(1-benzyl-3-indolylmethyl)malonate (3a).—A 500-ml. flask was charged with 250 ml. of liquid NH_3, 1.2 g. of 90% sodamide (0.0028 mole), and 8.65 g. (0.025 mole) of diethyl acetamido(3-indolylmethyl)malonate. The homogenous mixture was treated during 10 min. with 3.2 g. (0.015 mole) of benzyl chloride dissolved in 10 ml. of absolute ether and was stirred at room temperature until most of the ammonia had evaporated (*ca.* 3 hr.). The reaction flask was then swept with dry nitrogen to remove the last traces of ammonia. Methanol (10 ml.), acetic acid (10 ml.), ether (200 ml.), and water (100 ml.) were added to the flask and the mixture was stirred. The ether layer was separated and combined with the ether extracts (2) of the water layer. The combined ether solutions were washed with water, dried, and evaporated to dryness. The residue after recrystallization from 95% ethanol melted at 120–121°, yield 6.2 g. (57%). A second recrystallization did not change the melting point. A mixture of this preparation and diethyl acetamido(1-benzyl-3-indolylmethyl)malonate prepared according to Conforth, *et al.*,[6] melted at 120–121°.

Diethyl Acetamido(1-benzyl-2-methyl-3-indolylmethyl)-malonate (3b).—Diethyl acetamido(2-methyl-3-indolylmethyl)-malonate[14] (10.0 g., 0.0278 mole) was benzylated in the same manner as diethyl acetamido(3-indolylmethyl)malonate using 250 ml. of liquid NH_3, 1.35 g. of sodamide, and 3.8 g. of benzyl chloride. The crude gummy product obtained upon evaporation of the ethereal solution was dissolved in hot ethanol. The hot ethanolic solution was poured into ice–water, and the crystalline product was filtered off (yield 12.5 g., m.p. 137–141°) and dried azeotropically in benzene. The benzene solution was poured into 4 vol. of petroleum ether (b.p. 35–80°), and the precipitate (11.3 g.) was recrystallized from 90% ethanol; yield 7.5 g. (59.6%), m.p. 155–157°. A sample of the product, recrystallized again for analysis, melted at 158–160°.

Anal. Calcd. for $C_{26}H_{30}N_2O_5$: C, 69.31; H, 6.71; N, 6.22. Found: C, 69.26; H, 6.85; N, 6.13.

Diethyl Acetamido(1-benzyl-5-methoxy-3-indolylmethyl)-malonate (3c).—Benzylation of diethyl acetamido(5-methoxy-3-indolylmethyl)malonate[15] (11.6 g., 0.0308 mole) was carried out and worked up as described for the benzylation of diethyl acetamido(3-indolylmethyl)malonate. The hot ethanolic solution of the sirup obtained upon work-up of the reaction mixture was diluted with water until the first sign of turbidity and placed in a refrigerator to crystallize. The crystalline product (9.2 g., 64.2% yield) melted at 99–100°. A sample, recrystallized for analysis from ethanol, melted at 100–101°.

Anal. Calcd. for $C_{26}H_{30}N_2O_6$: C, 66.93; H, 6.48; N, 6.01. Found: C, 67.28; H, 6.86; N, 6.35.

DL-N-Acetyl-1-benzyl-2-methyltryptophan.—A mixture of 7.0 g. (0.0155 mole) of diethyl acetamido(1-benzyl-2-methyl-3-indolylmethyl)malonate (**3b**) and 31 ml. of 2.5 *N* NaOH was refluxed for 3 hr., cooled, acidified with 60 ml. of 2 *N* HCl, and refrigerated. The crystalline precipitate (4.7 g., 77.5% yield) melted at 105–112° and gave a satisfactory nitrogen analysis for the expected acetamidomalonic acid.

Anal. Calcd. for $C_{22}H_{22}N_2O_5$: N, 7.10. Found: N, 7.27.

The acetamidomalonic acid (4.7 g.) was decarboxylated by refluxing for 2 hr. with a mixture of 30 ml. of alcohol and 25 ml. of water. The hot reaction mixture was filtered, diluted with 200 ml. of water, and stored in the refrigerator overnight. The finely divided precipitate was centrifuged and recrystallized from about 65 ml. of 50% ethanol; yield 3.0 g. (72%), m.p. 213–214°. An analytical sample, obtained from a second recrystallization from alcohol, melted at 214–215°.

Anal. Calcd. for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 8.00. Found: C, 71.59; H, 6.46; N, 8.08.

(11) Microanalyses were carried out by Mr. John Deonorine of these Laboratories. All melting points were taken on a Thomas–Hoover melting point apparatus and are corrected.

(12) After the completion of our study, the preparation of this compound was described by H. Gershon and A. Scala, *J. Org. Chem.*, **26**, 2347 (1961), by a variation of our method. It was first prepared by A. Sanno, *Yakagaku Zasshi*, **78**, 1113 (1958); *Chem. Abstr.*, **53**, 5238 (1959), from either diethyl acetamido(2-piperdinomethyl)malonate, dimethyl sulfate, and 2,4-pentanedione or from diethyl acetamidomalonate, dimethyl sulfate, and 1,5-bis-(dimethylamino)-3-pentanone.

(13) R. Dahlbohm, *Acta Chem. Scand.*, **9**, 1074 (1955).

(14) H. N. Rydon, *J. Chem. Soc.*, 705 (1948).

(15) J. W. Cook, J. D. London, and P. McCloskey, *ibid.*, 1203 (1951).

DL-**N-Acetyl-1-benzyl-5-methoxytryptophan.**—A suspension of 7.5 g. (0.0161 mole) of diethyl acetamido(1-benzyl-5-methoxy-3-indolylmethyl)malonate (**3c**) in 32 ml. of 2.5 *N* NaOH was refluxed for 3 hr., clarified with charcoal, cooled to 5°, acidified with 50 ml. of 2 *N* HCl, and refrigerated overnight. The precipitate of acetamido(1-benzyl-5-methoxy-3-indolylmethyl)malonic acid (5.9 g., 92%) melted at 166–168°.

Anal. Calcd. for $C_{22}H_{22}N_2O_6$: N, 6.82. Found: 7.08.

A mixture of 5.5 g. (0.0136) of the acetamidomalonic acid, 100 ml. of ethanol, and 50 ml. of water was refluxed for 4 hr., distilled *in vacuo* to remove alcohol, and refrigerated overnight. The cream-colored precipitate of DL-N-acetyl-1-benzyl-5-methoxytryptophan was filtered off and dried (yield 4.4 g., 89.6%, m.p. 176–177°). A sample, recrystallized from 30% ethanol, melted at 178–179°.

Anal. Calcd. for $C_{21}H_{22}N_2O_4$: C, 68.83; H, 6.05; N, 7.65. Found: C, 69.11; H, 6.35; N, 7.70.

DL-**1-Benzyl-2-methyltryptophan (1b).**—DL-N-Acetyl-1-benzyl-2-methyltryptophan (2.9 g., 0.0083 mole) was refluxed for 24 hr. with 15 ml. of 2 *N* NaOH solution, treated with charcoal, filtered hot, and acidified to pH 5.5 with acetic acid. The crystalline precipitate was recrystallized from 50% ethanol; yield 0.45 g. (15%), m.p. 237–239°.

Anal. Calcd. for $C_{19}H_{20}N_2O_2$: C, 73.98; H, 6.54; N, 9.09. Found: C, 73.97; H, 6.21; N, 8.84.

DL-N-Acetyl-1-benzyl-2-methyltryptophan (1.2 g., m.p. 213° after recrystallization from ethanol) was recovered from the recrystallization mother liquor.

DL-**1-Benzyl-5-methoxytryptophan (1c).**—DL-N-Acetyl-1-benzyl-5-methoxytryptophan (3.0 g., 0.0082 mole) was refluxed with 14 ml. of 2 *N* NaOH for 25 hr., filtered hot, cooled, neutralized to pH 5.5 with acetic acid, and stored overnight in the refrigerator. The crystalline precipitate of crude **1c** was filtered off and recrystallized from 50% ethanol (yield 0.9 g., 33%, m.p. 232–233°). It melted at 229–230° after a second recrystallization from 50% ethanol.

Anal. Calcd. for $C_{20}H_{20}N_2O_3$: C, 70.35; H, 6.22; N, 8.64. Found: C, 70.18; H, 6.14; N, 8.54.

2-Amino-3-methylthiobutyric Acid, an Isoleucine Antagonist[1]

Tommy J. McCord, Drusilla Cook Howell, Don L. Tharp, and Alvie L. Davis

Department of Chemistry, Abilene Christian College, Abilene, Texas

Received December 10, 1963

2-Amino-3-methylthiobutyric acid, the thia analog of isoleucine, was prepared as a diastereoisomeric mixture by condensing 2-phenyl-4-ethylidene-5-oxazolidone and methanethiol in the presence of sodium methoxide, followed by acid hydrolysis of the resulting intermediate condensation product. 2-Amino-3-methylthiobutyric acid inhibits growth of *Escherichia coli*, *Streptococcus lactis*, *Leuconostoc dextranicum*, *Lactobacillus casei*, and *Leuconostoc mesenteroides* at concentration levels of 6, 6, 60, 60, and 60 γ/ml., respectively. A specific and competitive reversal of 2-amino-3-methylthiobutyric acid toxicity by isoleucine is observed with *E. coli* with an inhibition index of about 30 over a 100-fold range of increasing substrate concentrations.

Substitution of a sulfur atom for a methylene group in certain aliphatic amino acids has been successful in producing thia analogs which act as amino acid antagonists. Among such analogs, S-carbamoyl-L-cysteine (4-thiaglutamine) has been found to inhibit the growth of several lactobacilli by interfering with essential biological functions in which glutamine has a role, but its toxicity is only partially and noncompetitively reversed by glutamine.[2] S-(β-Aminoethyl)-L-cysteine (4-thialysine) has been found to antagonize competitively the utilization of lysine for the growth of *Leuconostoc mesenteroides* P-60 and *Lactobacillus arabinosus* 17-5.[3]

In view of the biological activity observed with these thia analogs, it was anticipated that the introduction of a sulfur in place of the methylene group at the 4-position of isoleucine might produce an effective antagonist of isoleucine in certain microorganisms. In this investigation, 2-amino-3-methylthiobutyric acid was prepared as a diastereoisomeric mixture, and its biological properties were studied in *Escherichia coli* 9723 and several lactobacilli.

Experimental[4]

Organic Syntheses. 2-Benzamido-3-methylthiobutyric Acid.—To a solution of 4.8 g. of methanethiol in 100 ml. of absolute methanol, in which 0.5 g. of sodium had reacted, was added slowly a solution of 18.5 g. of 2-phenyl-4-ethylidene-5-oxazolidone in 100 ml. of benzene at 5–10° with constant stirring. After addition was complete, the reaction mixture was allowed to stand at 40° for 72 hr. The reaction mixture was acidified to pH 2 by the addition of 6 *N* HCl and then taken to dryness by removal of the solvents under reduced pressure. The residual solid was extracted with 50 ml. of boiling ethanol, and the insoluble material was removed by filtration. After chilling the alcoholic filtrate in a refrigerator overnight, there was obtained 20.1 g. of white crystals, m.p. 72–74°. A sample recrystallized from hot ethanol melted at 74–75°.

Anal. Calcd. for $C_{12}H_{15}NO_3S$: C, 56.89; H, 5.97. Found: C, 56.97; H, 6.30.

2-Amino-3-methylthiobutyric Acid (4-Thiaisoleucine) Hydrochloride Monohydrate.—A solution of 2 g. of 2-benzamido-3-methylthiobutyric acid in 200 ml. of 4 *N* HCl was heated at reflux for 2 hr. The reaction mixture was extracted twice with 50-ml. portions of ether to remove the benzoic acid. The aqueous phase was taken to dryness *in vacuo*, and the residual oil was treated with 100 ml. of benzene. The resulting mixture was allowed to stand several days at room temperature in order to effect crystallization of the desired product. The solid was collected on a filter, washed with benzene, and dried in a desiccator under vacuum ($CaCl_2$). There was obtained 0.9 g. of chromatographically pure product which softened to a gelatinous solid at 65–67° and melted at 122–132° dec.

Anal. Calcd. for $C_5H_{11}NO_2S \cdot HCl \cdot H_2O$: C, 29.48; H, 6.92; N, 6.87; S, 15.74. Found: C, 29.71; H, 6.91; N, 7.20; S, 15.98.

The R_f values in 1-butanol–acetic acid–water (3:1:1), 65% pyridine, and 95% methanol were 0.50, 0.67, and 0.48, respec-

(1) The support of this work by Grant No. R-085 from the Robert A. Welch Foundation of Houston, Texas, is gratefully acknowledged.

(2) J. M. Ravel, T. J. McCord, C. G. Skinner, and W. Shive, *J. Biol. Chem.*, **232**, 159 (1958).

(3) T. Shiota, J. E. Folk, and F. Tietze, *Arch. Biochem. Biophys.*, **77**, 352 (1958).

(4) All melting points are corrected. The microanalyses were performed by International Chemical and Nuclear Corp., City of Industry, Calif. All R_f data were determined using the ascending technique of paper chromatography in the solvents indicated, and ninhydrin reagent was used for the development of the spots. The infrared spectrum was determined on a Beckman Instruments, Inc., Model IR-8 spectrophotometer using the potassium bromide pellet technique and at a concentration of 0.5%.

(5) H. E. Carter, P. Handler, and D. D. Melville, *J. Biol. Chem.*, **129**, 362 (1939).