(2) J. R. Bertino, ref 1, Part II, p 468.
(3) L. T. Plante, E. J. Crawford, and M. Friedkin, *J. Biol. Chem.*, **242**, 1466 (1967).
(4) J. R. Bertino, *Cancer Res.*, **39**, 293 (1979).
(5) M. Friedkin, E. J. Crawford, and L. T. Plante, *Ann. N.Y. Acad. Sci.*, **186**, 209 (1971).
(6) D. K. Mishra, S. R. Humphreys, M. Friedkin, A. Goldin, and E. J. Crawford, *Nature (London)*, **189**, 39 (1961).
(7) Carboxypeptidase G was obtained from the New England Enzyme Center, Tufts University School of Medicine, Boston, Mass. For a discussion of the properties of this enzyme, see J. L. McCullough, B. A. Chabner, and J. R. Bertino, *J. Biol. Chem.*, **246**, 7207 (1971).
(8) D. E. Wolf and K. Folkers, U.S. Patent 2515483; *Chem. Abstr.*, **44**, P8964*h* (1950).
(9) J. E. Martinelli, M. Chaykovsky, R. L. Kisliuk, Y. Gaumont, and M. C. Gittelman, *J. Med. Chem.*, **22**, in press (1979).
(10) T. Curtius and J. Jansen, *J. Prakt. Chem.*, **95**, 327 (1917).
(11) J. H. Bieri and M. Viscontini, *Helv. Chim. Acta.*, **56**, 2905 (1973).
(12) M. Viscontini and J. Bieri, *Helv. Chim. Acta.*, **54**, 2291 (1971); E. Khalifa, J. Bieri and M. Viscontini, *ibid.*, **56**, 2911 (1973). Interestingly, in the NMR spectrum one isomer may exhibit an A_2B_2 pattern while the other presents a singlet. Spectra in CF_3CO_2D and Me_2SO-d_6 are quite different.
(13) G. E. Foley and H. Lazarus, *Biochem. Pharmacol.*, **16**, 659 (1967).
(14) R. L. Kisliuk, D. Strumpf, Y. Gaumont, R. P. Leary, and L. Plante, *J. Med. Chem.*, **20**, 1531 (1977).
(15) H. H. W. Thijssen, *Anal. Biochem.*, **54**, 609 (1973).

Dipeptides of *O*-Methyl-L-threonine as Potential Antimalarials

Herman Gershon* and Ira S. Krull[1]

Boyce Thompson Institute for Plant Research at Cornell University, Ithaca, New York 14853. *Received February 5, 1979*

L-Leucyl-*O*-methyl-L-threonine, *O*-methyl-L-threonyl-L-leucine, and *O*-methyl-L-threonyl-*O*-methyl-L-threonine were prepared and compared with *O*-methyl-L-threonine and L-leucine for antimalarial activity against *Plasmodium berghei* in mice. *O*-Methyl-L-threonine significantly prolonged survival time at doses of 160, 320, and 640 mg/kg. *O*-Methyl-L-threonyl-*O*-methyl-L-threonine was less active, significantly prolonging survival time only at 640 mg/kg. L-Leucine, as well as the other two dipeptides, exhibited no activity in this test.

Since protozoans were known to have only limited capability for de novo synthesis of amino acids from simple sources, it is believed that the proteins of intraerythrocytic malarial plasmodia are made primarily from preformed amino acids of the host.[2] Existing evidence indicates that the free amino acid pools of red cells are too small to be significant and that most serum amino acids are not taken up by the plasmodium to form protein.[3,4] Serum proteins are also not utilized, and, therefore, the chief source of parasite protein must of necessity be derived from the hemoglobin of the red cells of the host.[2]

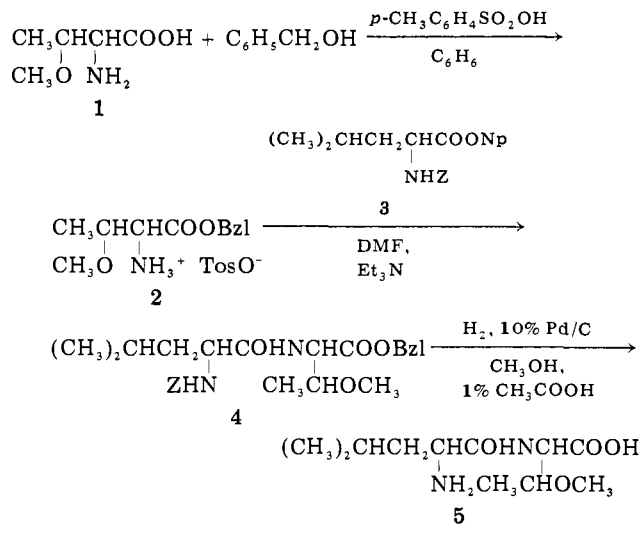
Electron microscopic studies of *Plasmodium lophurae* and *P. berghei* indicated that the parasites feed by phagocytosis of hemoglobin.[5,6] This was further demonstrated by growing *P. lophurae* in duck red cells containing radioactive hemoglobin. Radioactivity was recovered in the proteins of the parasite which were uncontaminated by host cell hemoglobin or parasite pigment.[7] Studies on the nutritional requirements of malarial parasites revealed that L-isoleucine was essential for the growth of the erythrocytic forms of *P. knowlesi*.[8,9]

It was shown that *O*-methyl-L-threonine [L-Thr(Me)], a known isoleucine antagonist,[10] strongly inhibited in vitro growth of *P. knowlesi*. Reversal was achieved with L-isoleucine, and growth repression was attributed to inhibition of protein synthesis.[11,12]

L-Isoleucine and L-leucine are structurally related, and a number of mutually antagonistic effects with these amino acids have been observed. A high L-leucine diet fed to rats results in marked reductions in L-isoleucine in the plasma. Loading human infants with L-leucine caused a marked[13] depression in L-isoleucine concentration in the plasma.[14]

A rational approach toward the control of the *Plasmodia*, causing malaria, may be made by interfering with the nutrition of the parasite. Since it was found that *P. knowlesi* requires L-isoleucine and hemoglobin is known to be a poor source of this amino acid, L-isoleucine must be obtained from the plasma of the host. The malaria-parasitized erythrocyte takes up 150 times as much L-isoleucine as the normal red cell.[15] The parasite thus appears to be vulnerable at several stages of its nutrition: proteolysis of the host's proteins, synthesis of its own proteins, and amino acid transport.

Scheme I. L-Leucyl-*O*-methyl-L-threonine [L-Leu-L-Thr(Me)] (5)

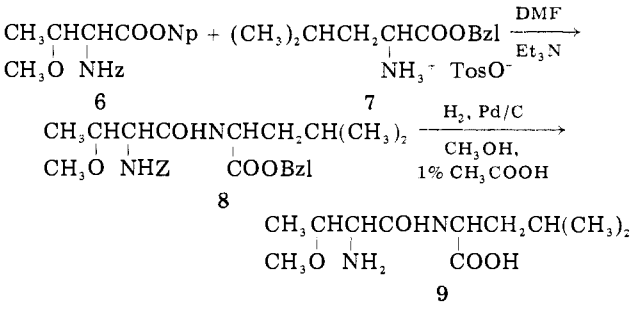


Np = *p*-nitrophenyl; Z = carbobenzoxy

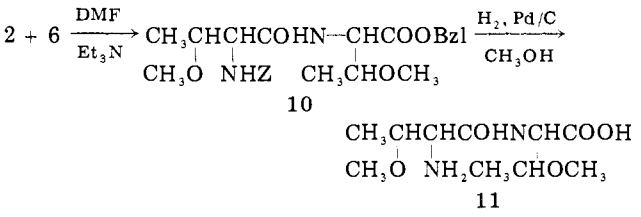
Pertinent facts concerning the role of peptides in biological systems were summarized by Meister.[16] Amino acids in peptide linkage are protected from destructive reactions that are specific for the corresponding free amino acids. Thus, gradual hydrolysis of a peptide can be a more efficient source of an amino acid than an equivalent quantity of the free amino acid. Certain peptides are transported across membranes more effectively than their constituent amino acids. When an amino acid antagonist exerts its effect on transport, peptides transported by another permeation mechanism would be expected to relieve the inhibition due to the antagonism, provided the

0022-2623/79/1822-0877$01.00/0

Scheme II. *O*-Methyl-L-threonyl-L-leucine [L-Thr(Me)-L-Leu] (9)



Scheme III. *O*-Methyl-L-threonyl-*O*-methyl-L-threonine [L-Thr(Me)-L-Thr(Me)](11)



peptide is hydrolyzed within the cell.

Thus, it appeared of interest to prepare three dipeptides of double antagonists, L-leucyl-*O*-methyl-L-threonine [L-Leu-L-Thr(Me)], *O*-methyl-L-threonyl-L-leucine [L-Thr(Me)-L-Leu], and *O*-methyl-L-threonyl-*O*-methyl-L-threonine [L-Thr(Me)-L-Thr(Me)] for antimalarial testing in *P. berghei* in mice.

The approach to the preparation of the dipeptides from L-Thr(Me)[17] (1) was carried out using standard methods in peptide chemistry (Schemes I–III).

For the preparation of L-Leu-L-Thr(Me) (5) (Scheme I), 1 was esterified with benzyl alcohol (BzlOH) in the presence of *p*-toluenesulfonic acid (TosOH) to form the tosylate salt of the ester (2).[18] Compound 2 was subsequently reacted with the 4-nitrophenyl (Np) ester of *N*-Z-L-leucine (3)[19] to yield the peptide 4, which upon hydrogenolysis using 10% Pd/C as the catalyst afforded 5.

The preparation of L-Thr(Me)-L-Leu (9) was achieved by condensing *N*-Z-*O*-methyl-L-threonine *p*-nitrophenyl ester (6)[20] with L-leucine benzyl ester *p*-toluenesulfonate salt (7).[20] The product 8 was freed of protective groups by hydrogenolysis over 10% Pd/C to yield 9 (Scheme II).

L-Thr(Me)-L-Thr(Me)[11] was prepared by condensing 2 with 6, followed by hydrogenolysis (Scheme III).

Compounds 5, 9, and 11 were tested for antimalarial activity in mice by the method of Rane.[21] Of these, only L-Thr(Me)-L-Thr(Me) (11) showed significant antimalarial activity. L-Thr(Me) significantly prolonged survival time at doses of 160, 320, and 640 mg/kg. L-Thr(Me)-L-Thr(Me) was less active, significantly prolonging survival time only at 640 mg/kg. L-Leu, as well as the other two dipeptides, exhibited no activity in this test and were not toxic below 640 mg/kg.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer Model 221 spectrophotometer. NMR spectra were recorded with a Varian XL 100 spectrophotometer (100 MHz). Thin-layer chromatography (TLC) was performed on Brinkmann silica gel F-254 plates (0.25 mm). Optical rotations were measured at the sodium D line (589 nm) on a Perkin-Elmer Model 141-M polarimeter.

***O*-Methyl-L-threonine Benzyl Ester *p*-Toluenesulfonic Acid Salt (2).** A mixture of 1[17] (1.7 g, 0.014 mol), *p*-toluenesulfonic acid monohydrate (3.23 g, 0.017 mol), and benzyl alcohol (9 mL) in benzene (100 mL) were heated under reflux for 4 h, using a Dean–Stark trap for removal of H_2O. After the theoretical amount of H_2O was obtained, the solvents were removed in vacuo. An additional 50 mL of benzene was added to the residue and again removed in vacuo. A small amount of ether was added to the residue to facilitate crystallization. The product was purified by crystallization from a mixture of ethyl alcohol, ether, and petroleum ether: yield 2.3 g (46%); mp 135.5–137.5 °C; $[\alpha]^{20}_D$ −27.3° (*c* 1.10, CH_3OH). Anal. ($C_{19}H_{25}NO_6S$) C, H, N, O, S.

***N*-Carbobenzoxy-L-leucyl-*O*-methyl-L-threonine Benzyl Ester (4).** A mixture of 2 (2.05 g, 0.0052 mol), *N*-carbobenzoxy-L-leucine *p*-nitrophenyl ester (3)[18] (2.01 g, 0.0052 mol), and triethylamine (0.53 g, 0.0052 mol) in DMF (8 mL) was stirred for 1.5 h at room temperature. The solvent was removed in vacuo, and 200 mL of H_2O was added to the residue. The aqueous solution was extracted several times with $CHCl_3$, and the $CHCl_3$ layer was washed successively with 2 × 50 mL of 5% $NaHCO_3$ and 1 × 100 mL of H_2O and dried (Na_2SO_4). After evaporation of the $CHCl_3$, an oily product was obtained which could not be crystallized or purified to yield a satisfactory elemental analysis but was useful for the next step.

L-Leucyl-*O*-methylthreonine (5). Impure 4 (24.0 g, 0.05 mol) was dissolved in 500 mL of CH_3OH containing 1% acetic acid, to which was added 300 mg of 10% Pd/C. The mixture was stirred vigorously and sparged with N_2 for 10 min. This was followed by H_2 which was added overnight. The reaction was complete when a test sample of gas yielded no precipitate when passed into $Ba(OH)_2$ solution. The system was flushed with N_2 for 10 min, the catalyst was removed by filtration, and the solvent was evaporated under reduced pressure. The residue was dissolved in 500 mL of H_2O, extracted with ether, and taken to dryness by reduced pressure distillation. The residue was crystallized from a mixture of ethyl alcohol and ether: mp 257–258 °C dec; $[\alpha]^{20}_D$ +19.7° (*c* 1.10, 1 N NaOH); TLC (silica gel) R_f in chloroform–methyl alcohol–28% aqueous ammonia (55:40:10) 0.05, in *n*-butyl alcohol–acetic acid–H_2O (9:5:1) 0.45 (spots visualized with ninhydrin); NMR [100 MHz (D_2O, K_2CO_3, DSS)] δ 4.20–4.10 (complex d, 1 H, $-C(CO_2H)HNHCO$), 4.07–3.94 (complex m, 1 H, $-C(NH_2)HCONH$), 3.94–3.75 (complex m, 1 H, $-C(OCH_3)-HCH$), 3.33 (s, 3 H, $-OCH_3$), 1.70–1.40 (complex m, 3 H, $-CH_2CH-$), 1.17–1.11 (d, 3 H, CH_3CH-), 0.98–0.96, 0.92–0.90 [2 d, 6 H, $-CH(CH_3)_2$]. Anal. ($C_{11}H_{22}N_2O_4$) C, H, N, O.

***N*-Carbobenzoxy-*O*-methyl-L-threonine *p*-Nitrophenyl Ester (6).** To a solution of carbobenzoxy-*O*-methyl-L-threonine[17] (4.7 g, 0.018 mol) in 100 mL of ethyl acetate was added *p*-nitrophenol (3.0 g, 0.0216 mol). The solution was cooled to 0 °C and dicyclohexylcarbodiimide (2.9 g, 0.014 mol) was added with stirring. After 0.5 h at 0 °C, the mixture was allowed to come to room temperature, at which it was kept for 1 h. The insoluble *N,N'*-dicyclohexylurea was removed by filtration and washed with ethyl acetate. The combined filtrates were evaporated to dryness, and the residue was crystallized from ethyl alcohol in 2.5 g (46%) yield, mp 71.5–72.5 °C. An analytical sample was crystallized from ether–petroleum ether (1:1), followed by vacuum drying at room temperature for 3 h: mp 71.5–72.5 °C; $[\alpha]^{20}_D$ −12.0° (*c* 2.0, DMF). Anal. ($C_{19}H_{20}N_2O_7$) C, H, N, O.

L-Leucine Benzyl Ester *p*-Toluenesulfonic Acid Salt (7). The title compound was prepared in the same manner as 2. The product was crystallized from a mixture of ethyl alcohol and ether and was obtained in nearly quantitative yield: mp 152–153 °C; $[\alpha]^{20}_D$ +8.3° (*c* 1.90, DMF) [lit. (22) mp 158.5–160 °C; $[\alpha]^{25}_D$ −1.7° (*c* 2%, CH_3OH)]. Anal. ($C_{20}H_{27}NO_5S$) C, H, N, O.

***N*-Carbobenzoxy-*O*-methyl-L-threonyl-L-leucine Benzyl Ester (8).** 6 (2.02 g, 0.0052 mol), 7 (2.05 g, 0.0052 mol), and triethylamine (0.53 g, 0.0052 mol) in DMF (8 mL) were reacted in the same manner as for the preparation of *N*-carbobenzoxy-1-leucyl-*O*-methyl-L-threonine benzyl ester (4). The yield of product was 1.8 g (74%). The analytical sample was crystallized from a mixture of ethyl acetate and petroleum ether, mp 57–59 °C. Anal. ($C_{26}H_{34}N_2O_6$) C, H, N.

***O*-Methyl-L-threonyl-L-leucine (9).** The hydrogenolysis of 8 to yield the title compound was carried out in the same manner as that for the preparation of 5. The yield of product was 60%,

and the analytical sample was crystallized from ethyl alcohol: mp 264–265 °C dec; $[\alpha]^{20}_D$ −36.5° (c 1.0, 1 N NaOH); TLC (silica gel) R_f in methyl alcohol 0.75, chloroform–methyl alcohol–28% aqueous ammonia (55:40:10) 0.80, n-butyl alcohol–acetic acid–H_2O (9:5:1) 0.50 (spots visualized with ninhydrin); NMR [100 MHz (D_2O, K_2CO_3, DSS)] δ 4.00–3.87 (complex d, 1 H, $-C(NH_2)-HCONH$), 3.70–3.40 (qd[a], 1 H $-C(OCH_3)H$), 3.30–3.10 (complex s, 3 H, $-OCH_3$), 2.90–2.75 (complex t, 1 H, $-NHC(CO_2H)H$), 1.70–1.35 (complex m, 3 H, $-CH_2CH-$), 1.30–1.10 (complex d, 3 H, CH_3CH-), 0.95–0.75 [complex d, 6 H, $-CH(CH_3)_2$]. Anal. ($C_{11}H_{22}N_2O_4$) C, H, N, O.

***N*-Carbobenzoxy-*O*-methyl-L-threonyl-L-threonine Benzyl Ester (10).** Compound **10** was prepared in 65% yield from **2**, **9**, and triethylamine in DMF in the same manner as *N*-carbobenzoxy-L-leucyl-*O*-methyl-L-threonine benzyl ester (**4**). The analytical sample was crystallized from an ethyl acetate–petroleum ether mixture, mp 105–106.5 °C. Anal. ($C_{25}H_{31}N_2O_7$) C, H, N.

***O*-Methyl-L-threonyl-*O*-methyl-L-threonine (11).** **10** was submitted to hydrogenolysis in the same manner as **5**. The yield of the product was 62%. An analytical sample was crystallized from ethyl alcohol: mp 224–225 °C dec; $[\alpha]^{20}_D$ −7.1° (c 1.10, 1 N NaOH); TLC (silica gel) R_f in chloroform–methyl alcohol–28% aqueous ammonia (55:40:10) 0.50, n-butyl alcohol–acetic acid–H_2O (9:5:1) 0.25 (spots visualized with ninhydrin); NMR [60 MHz (D_2O, K_2CO_3, DSS)] δ 4.33–4.25 (d, 1 H, $-C(CO_2H)HNHCO-$), 4.25–4.10 (d, 1 H, $-C(NH_2)HCONH-$), 4.10–3.80 (complex m, 2 H, $-C-(OCH_3)H-$), 3.45–3.41 (2 s, 6 H, $-OCH_3$), 1.33–1.22, 1.28–1.17 (2 d, 6 H, CH_3CH-). Anal. ($C_{10}H_{20}N_2O_5$) C, H, N, O.

Acknowledgment. This work was supported in part by the U.S. Army Medical Research and Development Command, Washington, D.C., under Contract DADA17-72-C2131. We thank Drs. T. Sweeney and E. A. Steck of the Walter Reed Army Institute of Research for making the antimalarial data available to us. Thanks are due to Professors R. Frank and D. D. Clarke for the 100-MHz NMR spectra. The expert technical assistance of Messrs. D. Gawiak and A. Grefig is gratefully acknowledged. This is contribution no. 1534 from the Army Research Program on Malaria.

References and Notes

(1) Present address: Thermo Electron Corp., Waltham, Mass. 02154.
(2) J. W. Moulder, "The Biochemistry of Intracellular Parasitism", The University of Chicago Press, Chicago, Ill., 1962.
(3) R. W. McKee and Q. M. Geiman, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **7**, 172 (1948).
(4) J. D. Fulton and P. T. Grant, *Biochem. J.*, **63**, 274 (1956).
(5) M. A. Rudzinska and W. Trager, *J. Protozool.*, **4**, 190 (1957).
(6) M. A. Rudzinska and W. Trager, *J. Biophys. Biochem. Cytol.*, **3**, 103 (1959).
(7) I. W. Sherman and L. Tanigoshi, *Int. J. Biochem.*, **1**, 635 (1970).
(8) H. Polet and M. E. Conrad, *Proc. Soc. Exp. Biol. Med.*, **127**, 251 (1968).
(9) W. A. Siddiqui, J. V. Schnell, and Q. M. Geiman, *Mil. Med.*, **134**, 929 (1969).
(10) M. Rabinovitz, M. E. Olson, and D. M. Greenberg, *J. Am. Chem. Soc.* **77**, 3109 (1955).
(11) H. Polet and M. E. Conrad, *Proc. Soc. Exp. Biol. Med.*, **130**, 581 (1969).
(12) H. Polet, M. E. Conrad, and C. F. Barr, *Mil. Med.*, **134**, 939 (1969).
(13) R. Tannous, Q. R. Rogers, and A. E. Harper, *Arch. Biochem. Biophys.*, **113**, 356, (1966).
(14) S. E. Snyderman, L. E. Holt, Jr., P. M. Norton, and E. Roitman, in "Protein Nutrition and Free Amino Acid Patterns", J. H. Leatham, Ed., Rutgers University Press, New Brunswick, N.J., 1968, p 19.
(15) G. J. McCormick, *Exp. Parasitol.*, **27**, 143 (1970).
(16) A. Meister, "Biochemistry of the Amino Acids", Vol. 1, 2nd ed, Academic Press, New York, N.Y., 1965, p 259.
(17) A. Chimiak and J. Rudinger, *Collect. Czech. Chem. Commun.*, **30**, 2592 (1965).
(18) H. Gibian and E. Schröder, *Justus Liebigs Ann. Chem.*, **642**, 145 (1961).
(19) M. Bodanzsky and V. Du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
(20) L. Zervas, M. Winitz, and J. P. Greenstein, *J. Org. Chem.*, **22**, 1515 (1957).
(21) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967). All of the Rane tests were carried out at the Leo Rane laboratory of the University of Miami, Fla., under contract with the U.S. Army Medical Research and Development Command.
(22) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", Vol. 2, Wiley, New York, N.Y., 1961, p 936.

Some 11-Substituted Tetrahydrocannabinols. Synthesis and Comparison with the Potent Cannabinoid Metabolites 11-Hydroxytetrahydrocannabinols

Raymond S. Wilson,[1]

Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

B. R. Martin, and W. L. Dewey*

Department of Pharmacology, Medical College of Virginia, Richmond, Virginia 23298. *Received September 8, 1978*

A series of compounds was prepared in which the 11-hydroxyl of 11-hydroxy-Δ^8-THC, the potent metabolite of Δ^8-THC, was replaced by a methyl, methoxy, amino, or acetamido group. All of the compounds tested produced behavioral changes in dogs, but only the methoxy compound has analgesic properties in mice. An isosteric oxime was inactive in mice.

The primary active constituents of marihuana, Δ^8- and Δ^9-THC, are rapidly metabolized in vivo to their respective 11-hydroxy derivatives (Chart I). The parent compounds and their 11-hydroxy metabolites have similar pharmacological profiles.[2,3] In a variety of tests in different species, including monkeys,[4] mice,[5] rats,[6] dogs,[7] and man,[8] the 11-hydroxy metabolites have been reported to be more potent than the parent compounds. However, in at least one experiment utilizing human subjects the potency of the 11-hydroxy-Δ^9-THC was the same as the parent Δ^9-THC.[9]

We were interested in the analgesic properties of cannabinoids and recently reported[10] that the 11-hydroxy metabolites were more potent in mice than the parent

0022-2623/79/1822-0879$01.00/0