placed in 18 mL of scintillation fluid [6.0 g of 2,5-diphenyloxazole, 0.2 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, 1400 mL of toluene, and 600 mL of MeOH], and counted in a Beckman LS-230 liquid scintillation spectrometer. In the absence of added drug, 1.05 mmol of [8-^{14}C]-ATP was incorporated into DNA during the 10-min incubation period.

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Diastereoisomers of 5,10-Methylene-5,6,7,8-tetrahydropteroyl-D-glutamic Acid

R. L. Kisliuk,* D. Strumpf, Y. Gaumont, R. P. Leary, and L. Plante

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111.
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The diastereoisomers of 5,10-methylene 5,6,7,8-tetrahydropteroyl-D-glutamate were resolved and tested as substrates and inhibitors of *Lactobacillus casei* thymidylate synthetase. No activity was observed. The compounds were neither growth factors nor inhibitors for *Lactobacillus casei*, *Streptococcus faecium*, or *Pediococcus cerevisiae*. 7,8-Dihydropteroyl-D-glutamate is 50% as active as 7,8-dihydropteroyl-L-glutamate (dihydrofolate) as a substrate for *L. casei* dihydrofolate reductase.

Studies with diastereoisomers of 5,6,7,8-tetrahydropteroyl-L-glutamate and derivatives (tetrahydrofolates) at carbon 6 (Figure 1) have shown that in some biological systems one configuration, *l*, is active whereas the other, *d*, is inert. (Since the absolute configurations at carbon 6 are not established, the configuration found in naturally occurring tetrahydrofolate derivatives is arbitrarily designated *l* and its enantiomer *d*.) Examples include the growth of *Pediococcus cerevisiae* on 5-formyl 5,6,7,8-tetrahydropteroyl-L-glutamate[1] and the reaction catalyzed by chicken liver 5,10-methenyltetrahydrofolate cyclohydrolase.[2] However, *d*-5,10-methylene-5,6,7,8-tetrahydropteroyl-L-glutamate inhibits *Escherichia coli* methylenetetrahydrofolate dehydrogenase[3] and *Lactobacillus casei* thymidylate synthetase.[4] Furthermore *d*-5,6,7,8-tetrahydrohomopteroyl-L-glutamate is a potent inhibitor of the growth of *L. casei*[5] and *Streptococcus faecium*,[6] the corresponding *l* form being a growth factor. In view of these varied effects of stereochemical configuration on biological activity, we prepared and tested the diastereoisomers of 5,10-methylene 5,6,7,8-tetrahydropteroyl-D-glutamate.

Results and Discussion

The pattern of elution of the diastereoisomers of methylene tetrahydropteroyl-D-glutamate from diethylaminoethyl cellulose columns corresponds to that observed for the corresponding L-glutamate derivatives.[7,8] The peak emerging from the column first could be assigned the *d* configuration at carbon 6 because it has a negative ellipticity at 285 nm (Figure 2) as does *d*-5,10-methylene tetrahydropteroyl-L-glutamate.[4] The ellipticity contributed by the D-glutamate residue at 285 nm is 20% that caused by the asymmetric center at carbon 6 and therefore does not interfere with this assignment. (The ellipticity values reported[4] for methylenetetrahydrofolate diastereoisomers were inadvertently 0.1 of the correct values[9]). The second peak emerging from the column is assigned the *l* configuration at carbon 6 because its ellipticity (Figure 2) corresponds to *l*-5,10-methylene tetrahydropteroyl-L-glutamate.[4] The ultraviolet absorption spectrum of both diastereoisomers was the same, consisting of a single peak at 295 nm expected for 5,10-methylenetetrahydrofolate.[7]

Enzymatic Tests. Neither diastereoisomer showed cofactor activity for *L. casei* thymidylate synthetase whether the enzyme was assayed spectrophotometrically[10] or by the release of ^{3}H from deoxyuridine 5′-phosphate [5-^{3}H][11] (Table I). The K_m for *l*-5,10-methylene tetrahydropteroyl-L-glutamate under the same conditions is 1.5×10^{-5} M.[4]

Neither diastereoisomer inhibited thymidylate synthetase (Table I). For comparative purposes *d*-5,10-methylene tetrahydropteroyl-L-glutamate is shown to produce detectable inhibition under similar conditions

Table I. Reduced Derivatives of Pteroyl-D-glutamate as Substrates and Inhibitors of *L. casei* Thymidylate Synthetase

No.	Compd	Concn, M	ΔOD/min	^3H released CPM	% inhibn
	A. Substrates				
1	*dl*-5,10-Methylene tetrahydropteroyl-L-glutamate	3×10^{-4}	0.030	71 000	
2	*dl*-5,10-Methylene tetrahydropteroyl-D-glutamate	7×10^{-5}	0.000		
3	*d*-5,10-Methylene tetrahydropteroyl-D-glutamate	4.5×10^{-5}	0.000	<2 000	
4	*l*-5,10-Methylene tetrahydropteroyl-D-glutamate	4.5×10^{-5}	0.000	<2 000	
	B. Inhibitors: incubations as in 1 plus				
5	*dl*-5,10-Methylene tetrahydropteroyl-D-glutamate	3.3×10^{-5}	0.030		0
6	*d*-5,10-Methylene tetrahydropteroyl-D-glutamate	2.4×10^{-5}	0.030		0
7	*d*-5,10-Methylene tetrahydropteroyl-L-glutamate	6.3×10^{-5}	*a*		14
8	*l*-5,10-Methylene tetrahydropteroyl-D-glutamate	1.3×10^{-5}	0.030		0
9	7,8-Dihydropteroyl-D-glutamate	9.2×10^{-5}	0.027		10
10	7,8-Dihydropteroyl-L-glutamate	1×10^{-4}	*a*		26

[a] Incubation conditions differed slightly in that (1) *l*-5,10-methylene tetrahydropteroyl-L-glutamate was present at 1.5×10^{-4} M rather than the *dl* compound at 3×10^{-4} M and (2) the uninhibited ΔOD was 0.020 rather than 0.030.

Table II. Reduced Derivatives of Pteroyl-D-glutamate as Substrates and Inhibitors of *L. casei* Dihydrofolate Reductase

No.	Compd	Concn, M	ΔOD/min	% inhibn
	A. Substrates			
1	7,8-Dihydropteroyl-L-glutamate	5×10^{-5}	0.032	
2	7,8-Dihydropteroyl-D-glutamate	5×10^{-5}	0.015	
	B. Inhibitors: incubations as in 1 plus			
3	*d*-5,10-Methylene tetrahydropteroyl-D-glutamate	1.5×10^{-5}	0.032	0
4	*l*-5,10-Methylene tetrahydropteroyl-D-glutamate	1.5×10^{-5}	0.032	0
5	7,8-Dihydropteroyl-D-glutamate	5×10^{-5}	0.025	22

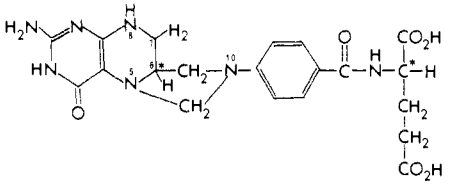

Figure 1. 5,10-Methylene-5,6,7,8-tetrahydropteroylglutamic acid. An asterisk indicates asymmetric carbons.

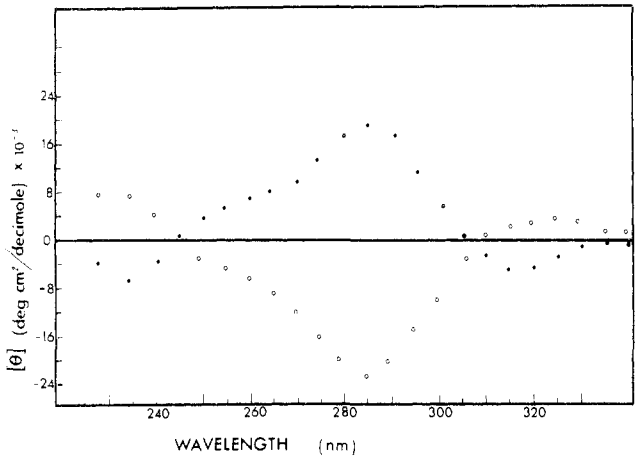

Figure 2. CD spectra of *l*-5,10-methylene tetrahydropteroyl-D-glutamate (●) and *d*-5,10-methylene tetrahydropteroyl-D-glutamate (○).

(Table I). An additional control was carried out with 10-methyl-5,8-deazafolic acid[12] synthesized in this laboratory. It inhibited the reaction 83% at 1.7×10^{-6} M, a similar level of potency to that found earlier with the *E. coli* enzyme.[13] 7,8-Dihydropteroyl-D-glutamate inhibited slightly (Table I). The corresponding L-glutamate derivative was more effective under similar conditions.

7,8-Dihydropteroyl-D-glutamate serves as a substrate for *L. casei* dihydrofolate reductase. It is 50% as active as the corresponding L-glutamate (Table II). As expected, it decreased the reaction rate when both substrates were present simultaneously (Table II, 5). Neither diastereoisomer of methylene tetrahydropteroyl-D-glutamate inhibited the reaction (Table II). The K_m for dihydropteroyl-L-glutamate is 3.6×10^{-7} M.[14]

Microbiological Test. Neither diastereoisomer of methylene tetrahydropteroyl-D-glutamate was a growth factor or inhibitor for *P. cerevisiae*, *L. casei*, or *S. faecium* at concentrations up to 5 μg/mL.

Our results are similar to those reported earlier[15] which showed 7,8-dihydropteroyl-D-glutamate to be 50% as active as the corresponding L derivative for mouse tumor dihydrofolate reductase. It was also shown[15] that *l*-5,10-methylene tetrahydropteroyl-D-glutamate showed little if any cofactor activity with *E. coli* thymidylate synthetase.

Thus, all of the tetrahydropteroyl-D-glutamate derivatives so far tested are inert; however, 7,8-dihydro-D-glutamate is a substrate for dihydrofolate reductase.

Experimental Section

Pteroyl-D-glutamic acid was a gift from Dr. R. B. Angier, Lederle Laboratories, Pearl River, N.Y. Its ultraviolet absorption spectrum was the same as that of pteroyl-L-glutamate[16] and its R_f (0.57) on Whatman No. 1 filter paper using 0.1 M NH_4HCO_3 as the solvent was also the same. Its CD spectrum was equal and opposite to that reported for pteroyl-L-glutamate,[17] the ellipticity at the peak (285 nm) being −5000 (deg cm^2)/dmol. *dl*-Tetrahydropteroylglutamate was prepared by catalytic hydrogenation in neutral aqueous solution.[18] The corresponding methylene derivatives were prepared by adding formaldehyde[19] and then separated on a diethylaminoethyl cellulose column. 7,8-Dihydropteroylglutamate was prepared by dithionite reduction.[20]

CD spectra were obtained using a Jasco J-20 recording spectropolarimeter. Thymidylate synthetase[10,11] and dihydrofolate reductase[21] incubations were carried out as described.

Substances assayed microbiologically were diluted in potassium ascorbate (6 mg/mL, pH 6.0) and added aseptically to the assay medium.[22] The final concentration of ascorbate in the assay was 0.6 mg/mL. *L. casei* (ATCC 7469) was grown on BBL folate assay broth (Becton, Dickinson and Co., Cockeysville, Md.) (folate 1 ng/mL), *P. cerevisiae* (ATCC 8081) on CF assay medium (Difco, Detroit, Mich.) (*dl*-5-formyltetrahydrofolate 1 ng/mL), and *S. faecium* (ATCC 8043) on the medium of Flynn et al.[23] (folate 1 ng/mL).

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Book Reviews

Biochemistry of Human Cancer. By Oscar Bodansky. Academic Press, New York, N.Y. 1975. xiv + 657 pp. 16 × 23 cm. $39.50.

From its earliest beginnings, biochemistry has had to deal with the cancer problem as a major issue. Almost a century ago the notion was put forward that there must be a fundamental metabolic difference between malignant and nonmalignant tissue, and the view persists even now that a cancer cure will be found by uncovering this difference and taking advantage of it to destroy tumor cells without harming "normal" ones. There are probably few medical chemists involved in the design and synthesis of antitumor agents today who do not accept this as a "central dogma" of their field. How fragile such an assumption may be is the theme of a scholarly and dispassionate new book by one of the world's authorities on human cancer biochemistry.

The first seven out of a total of 18 chapters in this excellent treatise are devoted to general aspects of the biochemistry of neoplastic disease. The rest focus on discrete tumor types and describe the many attempts that have been made over the years to relate tumors to specific metabolic or enzymic defects.

After a broad introductory chapter on the metabolism of proteins, lipids, and carbohydrates in cancerous vs. "normal" tissue, the reader is led through a meticulously arranged tour of the principal classes of enzymes—those concerned with glycolysis, those responsible for the cleavage of phosphate bonds, those involved in the breaking of amide and peptide bonds, and so on. This is followed by a chapter on immunoglobulins and the various immunoglobulinopathies associated with cancer (e.g., the existence of "Bence–Jones proteins" in the serum of patients with multiple myeloma). Two more chapters deal with the metabolism of tyrosine and tryptophan, a pair of amino acids for which cancer pathologists seem to have a special fascination. Tryptophan metabolites are implicated in the etiology of bladder cancer for example, whereas tyrosine and a plethora of catechols derived from tyrosine may be linked to neural crest tumors such as malignant melanoma and neuroblastoma.

The next 11 chapters, comprising approximately two-thirds of the total number of pages in the book, deal with specific tumor types and the biochemical correlates that pathologists sometimes rely upon in making a diagnosis.

In connection with tumors of the visceral organs, for example, may be cited the work of a number of investigators who believe that the serum of patients with colorectal carcinoma contains a unique glycoprotein, called carcinoembryonic antigen (CEA), which is absent in healthy subjects. Pancreatic tumors, also among the more common malignancies in the elderly, can be categorized with respect to their anatomical origin on the basis of certain biochemical criteria. Thus, one type of β-islet cell cancer, called insulinoma, appears to be correlated with hypoglycemia and elevated insulin production. Of course, hypoglycemia and a surplus of insulin do not in themselves signify that a tumor is present, and it is one of the virtues of this book that it stresses at every opportunity that such correlations mean very little in the absence of supporting histopathologic or clinical findings.

Leukemias and lymphomas, which are discussed together in a single chapter, occupy a special place among tumors because they often respond favorably to chemotherapy. Perhaps for this reason, more biochemical work seems to have been done with leukemic leukocytes than with other kinds of cancer cells. There is a detailed discussion of phosphatase and lysozyme levels in leukemic patients, but the most extensive coverage in this particular chapter is given to the labyrinthine complexities of purine and pyrimidine metabolism and nucleic acid biosynthesis in normal vs. leukemic cells. This discussion is particularly significant in relation to the widespread use of antimetabolites to arrest tumor growth.

Other chapters deal with bone tumors and associated disturbances in calcium and phosphorus metabolism, endocrine tumors and their hormonal correlates (e.g., the elevation of calcitonin in thryoid carcinoma), and tumors of the male and female reproductive organs. A very interesting chapter deals separately with a peculiar group of diseases called "ectopic syndromes". The word "ectopic" means "inappropriate" and refers to the fact that biochemical defects (especially those of a hormonal nature) are sometimes observed which are not those one would expect for a particular type of tumor. For instance, serum ACTH is sometimes elevated in patients whose pituitary gland shows no sign of malignancy but who turn out to have bronchial carcinoma. This would be referred to as an "ectopic