THE RELATIVE CONFIGURATION OF N^5-METHYL-L-TETRAHYDROFOLIC ACID

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1. Introduction

Tetrahydrofolic acid, the coenzyme of biochemical one-carbon reactions, may be prepared by either enzymatical or chemical reduction. Enzymatically, a single product is obtained, whereas by reduction with catalytically activated hydrogen or with alkali boranate, the two possible diastereomers are formed possessing different configurations at C_6 of the pterin moiety which has become chiral by reduction, but having in common the asymmetric α-carbon of the glutamic acid residue. Generally diastereomers may be resolved by chromatography but hitherto only the separation of *d*- and *l*-N^5,N^{10}-methylene-L-tetrahydrofolic acids (CH_2-FH_4) has been reported [1]. In the present work we describe the preparation of the optically pure isomers of *d,l*-N^5-methyl tetrahydrofolate. The resolution of diatereomers was performed at the level of N^5, N^{10}-methylene tetrahydrofolic acid. Rotational values of both methylene derivatives are reported. After reduction to the N^5-methyl compounds, only the dextrorotatory isomer is active in methionine biosynthesis [2].

2. Methods and results

On ion exchange chromatography of chemically prepared CH_3-FH_4 on TEAE-cellulose, the relative amount of biologically active CH_3-FH_4 increases slightly but significantly (fig. 1). A limited separation must have occurred by this method, though the effect was too small to be used for preparative purposes.

If, however, the diastereomers are resolved on TEAE-cellulose at the level of the N^5,N^{10}-methylene derivatives by a modification of the procedure of

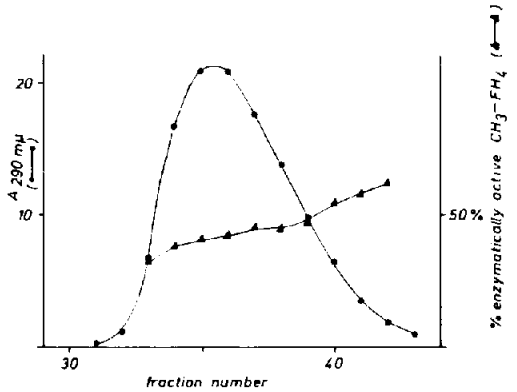

Fig. 1. Chromatography of *d,l*-CH_3-FH_4. 20 μmoles of *d,l*-CH_3-FH_4 were applied to a column of TEAE-cellulose (1 × 36 cm), and eluted with 0.15 M NH_4HCO_3, 0.05 M mercaptoethanol, pH 7.6. Fractions of 4 ml/30 min were collected. Biochemically active CH_3-FH_4 was determined by enzymatic demethylation, followed by formylation [3].

Kaufman, Donaldson, and Keresztesy [1], both isomers separate completely, the laevorotatory one preceding the dextrarotatory (fig. 2). However, we were not able to confirm the optical rotational values for both isomers, reported by others, namely $[\alpha]_{589}$ = −82° and +165° [5], or −97° and +142.5° [6], respectively. We found rotations differing predominantly in sign but not very much in absolute value. Using the Drude equation [7] and assuming a molecular weight of 453 daltons and $\epsilon_M^{290} = 2.5 \times 10^4$, we calculated specific rotations of $[\alpha]_{589} = -106°$ and +83° from the rotations measured at 546 and 578 nm in a filter polarimeter. These specific rotations correspond to molar rotations of $[M]_{589} = -478°$ and

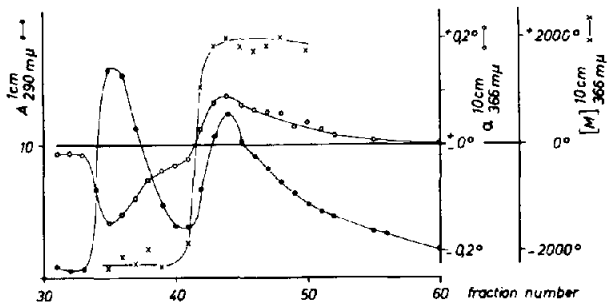

Fig. 2. Chromatography of *d,l*-CH_2-FH_4. 120 μmoles of *d,l*-CH_2-FH_4 were applied to a column of TEAE cellulose (3.2 × 34 cm) and eluted with 0.4 M NH_4HCO_3, 0.05 M mercaptoethanol, 0.02 M formaldehyde, pH 7.6. Fractions of 19 ml/30 min were collected. Rotational values α were determined in 10 cm cuvettes in a filter polarimeter (Carl Zeiss, Oberkochen/W.). Molar rotations [M] were calculated according to [4].

+376°, respectively [4]. Since normally rotational values at lower wavelengths are assayed more accurately than those at higher ones, fig. 2 shows degrees of rotation and molar rotations at 366 nm. For the laevorotatory CH_2-FH_4 of the first peak, we calculated an average molar rotation of $[M]_{366} = -2200 \pm 65°$, for the second peak $-1860 \pm 39°$. It seems unlikely that the diastereomers contaminate each other, since molar rotational values are constant over the whole range of both peaks. Furthermore, a decomposition of the compounds during separation can be excluded because the rotational values remain constant under chromatographic conditions, i.e. 4° C, pH 7.6, in the presence of mercaptoethanol and formaldehyde, for at least one day. At −20° C, they are stable for several months. Obviously, the rotational values are composed of the contribution of the pterin antipodes and of the L-glutamic acid residue, the latter one being slightly negative at alkaline pH values [4]. One should expect an additivity of rotational values because the distance between both asymmetric carbon atoms in CH_2-FH_4 is too far to allow a strong interaction. Possibly the dissimilar rotational values reported for both isomers [5, 6] reflect a dependence of optical rotation on ionic strength and pH value, since the previous authors used salt gradients for column elution.

The less basic dextrorotatory isomer of CH_2-FH_4 has been reported to be the substrate of methylene tetrahydrofolate dehydrogenase [1, 5, 6] and the parent substance of a microbiologically active CH_3-FH_4 which also has been isolated from horse liver ("Prefolic A") [1]. The folate dependent methionine synthetase (B_{12} dependent homocysteine N^5-methyl-tetrahydrofolate methyltransferase) from *Escherichia coli* [2] displays the same substrate specificity as methylene tetrahydrofolate dehydrogenase. We reduced both methylene diastereomers separately with sodium boranate [8], fractionated the products on a column of Sephadex G-10 and assayed their activity in methionine biosynthesis [3]. Table 1 shows that CH_3-FH_4 generated from the less basic *d*-methylene compound is the natural isomer. The product generated from the *l*-methylene derivative is nearly inactive in the enzymatic reaction. The small residual activity may be derived from a partial racemization due to a transient oxidation of the tetrahydropterin, followed by a new reduction by boranate. The comparatively low activity of the natural isomer may have an analogous explanation. The mixture of the diastereomers displays half the activity expected from optical density measurements, so it may be concluded that the unnatural isomer does not inhibit the enzymatic transmethylation.

Table 1

Enzymatic activities of N^5-methyl tetrahydrofolates. Material from either peak in fig, 2 was collected, concentrated *in vacuo*, reduced with sodium boranate [7] and passed through a column of Sephadex G-10 (1 × 30 cm) with 0.01 M sodium phosphate buffer, pH 7.0, as eluant.

Substance	% enzymatically active
Material from peak I (fig. 2)	9.0
Material from peak II (fig. 2)	66.5
d,l-Methyl-L-tetrahydrofolate	48.8

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