Biochemical Pharmacology, Vol. 28, pp. 2219 - 2222.

0006-2952/79/0715-2219 $02.00/0

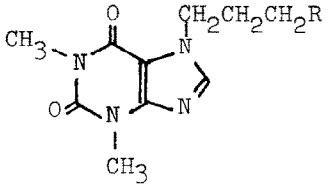
N^5,N^{10}-METHYLENE TETRAHYDROFOLIC ACID AS A FORMALDEHYDE DONOR IN DRUG BIOTRANSFORMATION

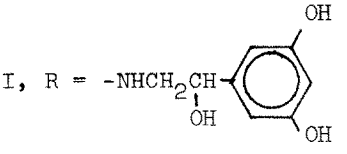
N. Kucharczyk, J. Yang and R.D. Sofia

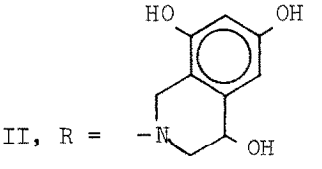
Wallace Laboratories
Division of Carter-Wallace, Inc.
Cranbury, New Jersey 08512

(Received 19 March 1979; accepted 16 April 1979)

The bronchodilator reproterol*, 7-{3-[2-(3,5-dihydroxyphenyl)-2-hydroxyethylamino]propyl}theophylline, hydrochloride (**I**) was reported to be metabolized to one major metabolite in all species[1]. The structure of this metabolite was identified to be that of 7-[3-(4,5,8-trihydroxy-1,2,3,4-tetrahydroisoquinolinyl-2)propyl]theophylline (**II**)[2]. Its formation *in vivo* assumes addition of formaldehyde to the parent molecule and cyclization to the corresponding tetrahydroisoquinoline. This type of metabolic conversion





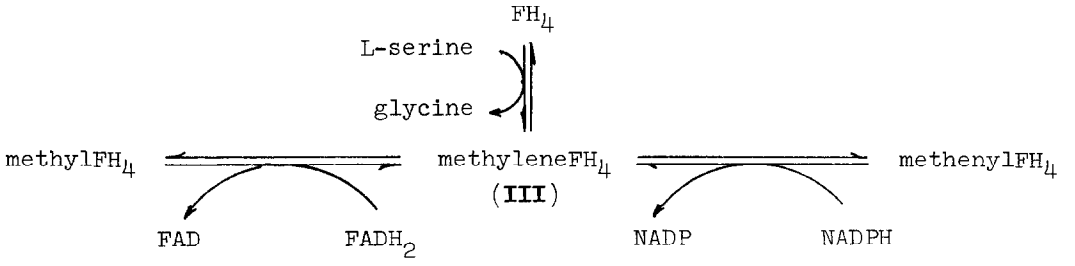


involving formaldehyde (analogous to the Pictet-Spengler synthesis) has been described for the endogenous substances dopamine and tryptamine and recently reviewed[3]. The formation of products of other aldehydes such as acetaldehyde with catecholamines has been also reported[4,5,6] and their effects on tyrosine hydroxylase activity of the brain have been studied[7].

One of the likely sources of the formaldehyde needed for these biotransformation reactions could be the so called "active formaldehyde" of N^5,N^{10}-methylene tetrahydrofolic acid (**III**)[8]. This coenzyme is known to

*Bronchospasmin^R, property of Chemiewerk Homburg, Degussa, Frankfurt/Main.

take part in many enzymatic one-carbon group transfer reactions[9], such as thymidylate and α-methylserine synthesis and is also formed in a complex process of glycine decarboxylation. It can be generated by at least three major reversible reactions of the folate pathway as shown below.



The cellular concentration of tetrahydrofolates was suggested to be regulated through enzyme feedback mechanisms within the pathway[10].

The involvement of **III** in the metabolism of trans-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol to yield 6,8-dibromo-3-(trans-4-hydroxycyclohexyl)-1,2,3,4-tetrahydroquinazoline was assumed by Jauch et al.[11], who showed that the label from L-[^{14}C]methylmethionine fed to rabbits was incorporated into the metabolite.

We have now established that reproterol is converted to **II** by interaction with **III**, generated enzymatically in vitro from D,L-tetrahydrofolic acid (**IV**) and L-serine in the presence of rat liver mitochondrial serine hydroxymethyltransferase (EC 2.1.2.1)[12]. The following procedure was used: Four ml of a mixture of 1 mg **IV** (1.8 μmol), 1 mg L-serine (9.5 μmol) and rat liver mitochondria (20.5 mg protein) in 0.02 M potassium phosphate buffer, pH 7.3, was incubated under nitrogen at room temperature (22°C) for 30 min. A sample (0.5 ml) was taken for analysis and to the remaining solution was added 0.4 mg **I** (0.94 μmol) in 0.5 ml buffer. Periodically 0.5 ml subsamples were taken, deproteinized with 0.5 ml methanol and centrifuged. They were analyzed by HPLC on a μBondapak C_{18} reversed-phase column, the solvent being 13% acetonitrile in 0.01 M sodium acetate buffer, pH 4.0, and the U.V. detector at 280 nm (HPLC system 1). The retention times (min) were: **IV** - 5.4, **III** - 11.6, **I** - 13.0, **II** - 14.6. After 0.5, 1, 2 and 3 h the amount of **I** converted to **II** was 28, 47, 72 and 87%.

When labeled D,L-[3-^{14}C]serine was used in the incubation mixture, 12.7% of the label which was stoichiometrically possible to be incorporated was recovered in the metabolite **II** fraction. We found that formation of **II**

from **I** did not require the addition of L-serine. This indicated the presence of other suitable substrates for the enzymatic formation of **III** in mitochondria. No significant conversion was observed by incubation without **IV** or mitochondria. The incorporation of the radioactivity, originating from the hydroxymethyl group of L-serine, is good evidence that **III** was the donor of formaldehyde needed for the formation of **II** from **I**.

To determine if the hydroxymethyltransferase is also essential for this reaction, pure **III** was needed. The following procedure was found to be suitable: To 2 ml of a solution of **IV** (5 mg, 9.2 μmol) in phosphate buffer, pH 7.3, 25 μl of a 0.37% formaldehyde solution (0.0925 mg, 3.1 μmol) was added under nitrogen and kept for 10 min in an ice bath. An 8 μl sample of this solution (equivalent to 20 μg of **IV**, 0.037 μmol) was analyzed by HPLC on a μBondapak C_{18} column with 4% acetonitrile in 0.01 M ammonium monohydrogen phosphate, pH 7.9, as the mobile phase (system 2). The peak corresponding to **III** was collected into a solution of **I** (12 μg, 0.028 μmol) under nitrogen, allowed to stand at room temperature for 1 h and reanalyzed by HPLC in system 1 (Figure 1,d). At pH 7.9 the amount of **I** converted to **II** was 66 and at pH 7.0 89%. Under the conditions of HPLC system 2, **III** was sufficiently stable (Figure 1,c). After the reaction of **III** with **I** an unknown peak was observed.

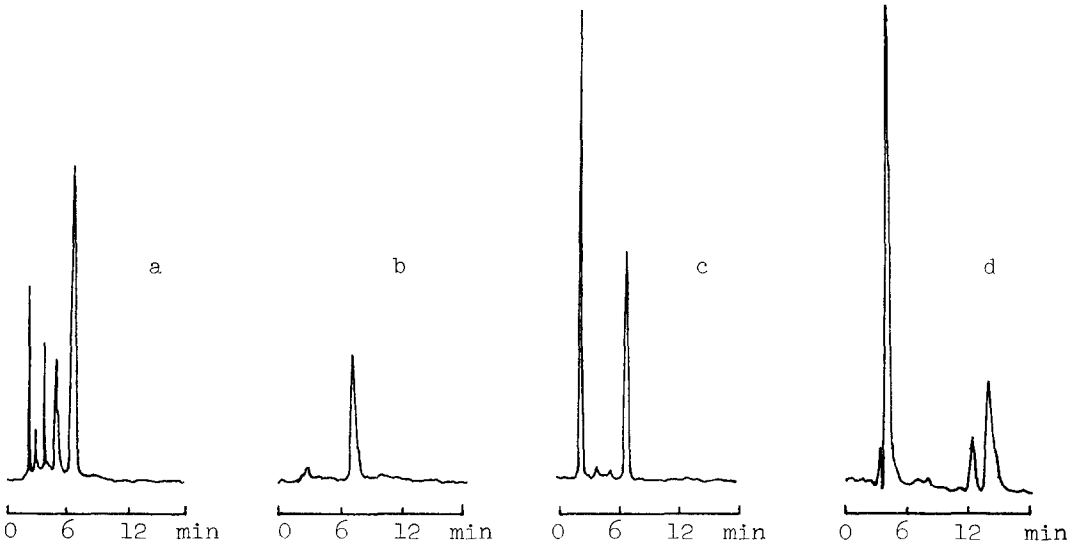


Figure 1. HPLC of a) reaction mixture of **IV** and formaldehyde, b) fraction of pure **III** before and c) after reaction with **I**. System 2 retention times (min): **IV** - 3.0, **III** - 7.1, unknown - 2.2. Compounds **I** and **II** are not eluted from the column in system 2 and were reanalyzed d) in system 1, retention time (min): **I** - 13.0, **II** - 14.6.

Our results demonstrate that the coenzyme **III** can indeed react under physiological conditions with **I** to yield **II**. No catalytic enzyme activity seems to be required for this reaction. The chemical reactivity of **III** is in agreement with the findings of Osborn et al.[8] as well as with the study on the mechanism of condensation of formaldehyde and **IV** carried out by Kallen and Jencks[13].

The potential of other compounds, similar in structure (1-phenyl-2-aminoethanols, catecholamines), to undergo this reaction is under investigation.

References:

1. G. Niebch, K. Obermeier, H. Vergin, K. Thiemer, Arzneim.-Forsch./Drug Res. 27 (1), 37 (1977).
2. G. Niebch, K.H. Klinger, G. Eikelmann, N. Kucharczyk, Arzneim.-Forsch./Drug Res. 28 (I), 765 (1978).
3. A.J. Turner, Biochem. Pharmac. 26, 1009 (1977).
4. M.A. Collins, M.G. Bidgeli, Life Sci. 16, 585 (1975).
5. G. Cohen, Biochem. Pharmac. 25, 1123 (1976).
6. T.M. Kenyhercz, P.T. Kissinger, J. Pharm. Sci. 67, 112 (1978).
7. C.D. Weiner, M.A. Collins, Biochem. Pharmac. 27, 2699 (1978).
8. M.J. Osborn, P.T. Talbert, F.M. Huennekens, J. Am. Chem. Soc. 82, 4921 (1960).
9. J.I. Rader, F.M. Huennekens, in The Enzymes (Ed. P.O. Boyer), p. 209. Academic Press, New York (1973).
10. P.B. Rowe, G.P. Lewis, Biochemistry 12, 1962 (1973).
11. R. Jauch, G. Bozler, R. Hammer, F.W. Koss, Arzneim.-Forsch./Drug Res. 28, 904 (1978).
12. Y. Nakano, M. Fujioka, H. Wada, Biochim. Biophys. Acta. 159, 19 (1968).
13. R.G. Kallen, W.P. Jencks, J. Biol. Chem. 241, 5851 (1966).