SYNTHESIS OF TRITIUM AND OF DEUTERIUM LABELLED PTERIDINES OF HIGH ACTIVITY

Heinz Rembold* and Günter Hennings

Max-Planck-Institut für Biochemie, D-8033 Martinsried, F. R. G.

Adolf Butenandt at the occasion of his 75th birthday

2,4-Diamino-6,7-dimethylpteridine and 2-amino-4-hydroxy-6,7-dimethylpteridine were synthetized with high specific ^{3}H and ^{2}D labelling in the 6,7- and the 7-methyl groups. The isotopes are stable in these positions. No HT- or HD-exchange could be observed.

Reduced pteridines are cofactors of certain mixed function oxygenases like phenylalanine, tyrosine, or tryptophan hydroxylase, and they may be involved in mitochondrial electron transfer [1]. From effects of parenteral tetrahydrobiopterin application in atypical phenylketonuria[2] it has been concluded, that intracellular pteridine concentrations are strongly dependent on their plasma concentration. But the quantity of cofactor is too low in blood as to allow its direct measurement. Only after synthesis of tritium-labelled pteridines of high specific radioactivity, studies on pteridine-protein interactions [3] and on the renal balance of the pterin cofactor [4] have become possible.

For the synthesis of 2,4-diamino-6,7-tritiomethyl-pteridine (6,7-^{3}H-DADMPt) and of 2-amino-4-hydroxy-6,7-tritiomethyl-pteridine (6,7-^{3}H-DMPt), 1,4-dibromobutane-2,3-dione was condensed with 2,4,5,6-tetraaminopyrimidine and 2,5,6-tetraamino-4-hydroxypyrimidine, respectively, to the 2,4-diamino-6,7-bromomethyl-pteridine and the corresponding 2-amino-4-hydroxy-6,7-bromomethyl-pteridine, following established methods [5,6]. For catalytic bromo-tritium-exchange, 5 mg of the bromomethyl-pteridine and 3 mg of the catalyst (10 % Pd/$CaCO_3$) were suspended in 2 ml of dry tetrahydrofuran in a closed 2.5 ml vessel, which was cooled in liquid nitrogen and then was evacuated. After warming up to room temperature, 2.5 Ci of carrier-free tritium gas was added through a manifold and the suspension was vigorously stirred for 6 hours. After separation from tritium gas, the crude pteridine suspension was dissolved in 20 ml of 0.1 N HCl and evaporated to dryness. To remove labile tritium, 150 ml of methanol were added to the residue, the solvent was removed again and this procedure was repeated for three times. Then the dry substance was dissolved in 5 ml of 0.1 N HCl, applied to TLC plates (Merck No. 5733) and then chromatographed with butanol, glacial acetic acid, water (4:1:1, v/v). The pteridine band was eluted and finally chromatographed on phosphocellulose. Purity was checked in five solvent systems by TLC as shown in the table. Specific radioactivity remained constant and was for (6,7-^{3}H)DADMPt = 5.3 and for (6,7-^{3}H)DMPt = 3.9 Ci · mmol^{-1}. 2-Amino-4-hydroxy-6-methyl-7-tritiomethyl-pteridine was synthetized via direct bromination of the 7-methyl group of 6,7-dimethylpterin [5] and subsequent cata-

Table. R_f values of 2,4-diamino-6,7-tritiomethyl-pteridine (6,7-^{3}H-DADMPt) and of 2-amino-4-hydroxy-6,7-tritiomethyl-pteridine (6,7-^{3}H-DMPt) on Silica Gel 60 TLC (Merck No. 5721). Solvent systems (v/v). A: n-butanol, acetic acid, water (4:1:1); B: water, 3 % NH_4Cl; C: water, 4 % Na-citrate; D: n-propanol, 1 % ammonia (2:1); E: propanol-2, 5 % H_3BO_3 (4:1); F: methanol, water (4:1); G: dioxane, 5 % ammonia (76:24)

solvent	A	B	C	D	E	F	G
6,7-^{3}H-DADMPt	0.49	0.78	0.78	0.28	0.55	0.73	0.52
6,7-^{3}H-DMPt	0.34	0.67	0.60	0.26	0.25	0.67	0.32

lytic bromo-tritium-exchange as already described. After purification the compound had a specific radioactivity of 3.3 Ci · $mmol^{-1}$.

2,4-Diamino-6,7-trideuteromethyl-pteridine (6,7-$^2D_3$-DADMPt) and 2-amino-4-hydroxy-6,7-trideuteromethyl-pteridine (6,7-$^2D_3$-DMPt) were synthetized by condensation of hexadeuterobutane-2,3-dione [7] with the corresponding pyrimidine derivatives. Rapid HD- and HT-exchange of the 7-methyl protons of 2,4-dihydroxy-6,7-dimethyl-pteridine in aqueous solutions has recently been reported by Plaut *et al.* [8,9]. *This is not the case with our labelled* 2,4-diamino- and 2-amino-4-hydroxy-pteridines due to kinetic NMR measurements and to mass spectrometry. Moreover, specific radioactivity of tritiated pteridines remained constant during cry-

stallization and chromatography. The 4-amino group of 2,4-diamino-6,7-dimethyl-pteridine was hydrolyzed to an extent of about 5 % during storage at -20°C under nitrogen for six months, with the corresponding 6,7-dimethylpterin as main product.

ACKNOWLEDGEMENT We are indepted to Dr. P.E. Schulze, Schering AG, Berlin, for his generous help in the synthesis of the tritiated pteridines.

REFERENCES

1 H. Rembold and W.L. Gyure, Angew. Chem. Int. Ed., 1972, 11, 1061.

2 D.M. Danks, R.G.H. Cotton, and P. Schlesinger, Lancet, 1975, II, 1043.

3 H. Rembold, K. Buff, and G. Hennings, Clin. Chim. Acta, 1977, 76, 329.

4 D.A. Häberle, H. Schiffl, G. Mayer, G. Hennings, and H. Rembold, Pflügers Arch., 1978, 375, 9.

5 J.H. Boothe, J.M. Mowat, G.W. Waller, R.B. Angier, J. Semb, and A.L. Gazzola, J. Amer. Chem. Soc., 1952, 74, 5407.

6 C.W. Waller, M.J. Fahrenbach, J.H. Boothe, R.B. Angier, B.L. Hutchings, J.H. Mowat, J.F. Poletto, and J. Semb, J. Amer. Chem. Soc., 1952, 74, 5405.

7 D.S. Herr, M.S. Matheson, and W.D. Walters, J. Amer. Chem. Soc., 1941, 63, 1464.

8 R.L. Beach and G.W.E. Plaut, Biochem., 1970, 9, 760.

9 G.W.E. Plaut, R.L. Beach, and T. Aogaichi, Biochem.,1970,9,771.

Received, 19th December, 1978