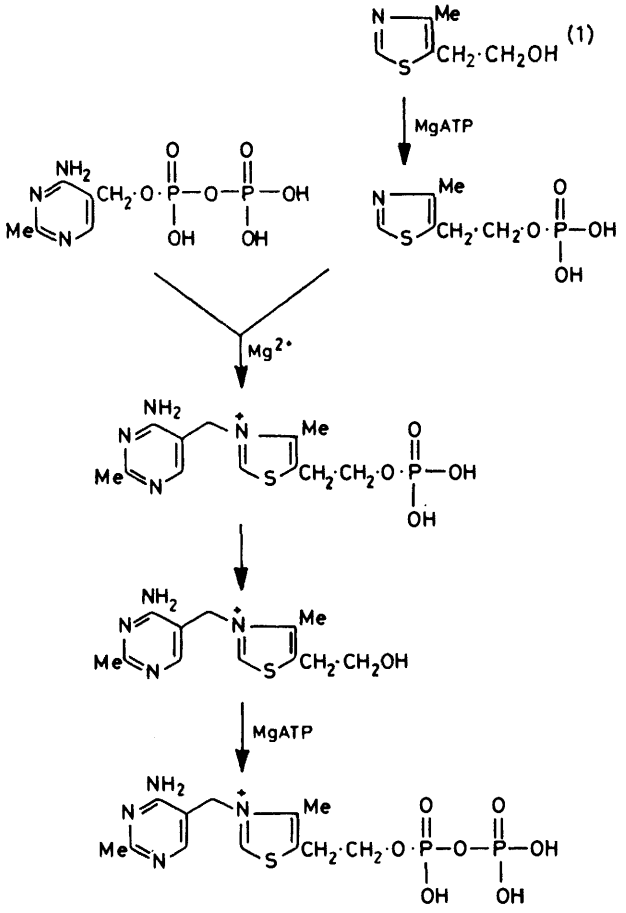
Bacteriostatic Activity of Fluoro-analogues of 5-(2-Hydroxyethyl)-4-methylthiazole, a Metabolic Intermediate in the Biosynthesis of Thiamine

By **Gordon Lowe** * and **Barry V. L. Potter**, The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY

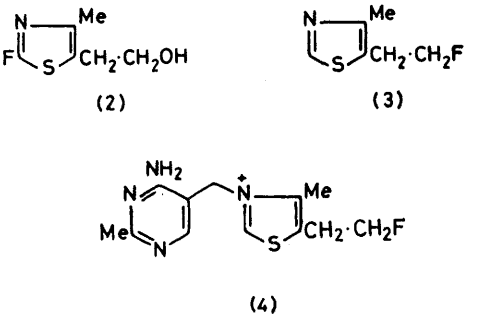
2-Fluoro-5-(2-hydroxyethyl)-4-methylthiazole and 5-(2-fluoroethyl)-4-methylthiazole have been prepared and the latter converted into a fluoro-analogue of thiamine. All three compounds show bacteriostatic activity against *E. coli*.

THE biosynthetic pathways leading to the vitamins provide a variety of potential targets for antimicrobial agents which are non-toxic to man. Although there are notable examples of this type of therapeutic agent, for example, the sulphonamides as inhibitors of folic acid biosynthesis,[1] this approach to new antibiotics remains relatively unexplored. Thus, although numerous analogues of thiamine and its precursors have been synthesised in relation to vitamin activity,[2] their potential as



SCHEME The later stages in the biosynthesis of thiamine pyrophosphate

antimicrobial agents has been largely ignored. Exceptions are 4-amino-5-hydroxymethyl-2-methylthiopyrimidine, methioprim,[3] and the corresponding 2-methoxy-derivative, bacimethrin.[4] Thiamine thiazone pyrophosphate is a potent inhibitor of pyruvate dehydrogenase from *E. coli*,[5] while 2-amino-5-(2-hydroxyethyl)-4-methylthiazole inhibits phosphorylation of the thiazole intermediate in thiamine biosynthesis.[6,7] The



later stages in the biosynthesis of thiamine pyrophosphate are outlined in the Scheme.[8]

Thiamine pyrophosphate is a co-factor for enzymes which decarboxylate α-keto-acids.[9] An essential feature for its activity is the lability of the C-2 proton of the thiazolium ring, the carbanion so formed adding to the ketone of the substrate to initiate decarboxylation.[10] If the C-2 position is blocked by a group of similar steric requirement, for example fluorine,[11,12] the co-enzyme would be ineffective and possibly a potent active-site-directed inhibitor.

2-Fluoro-5-(2-hydroxyethyl)-4-methylthiazole (2) was considered to be a good structural analogue of the natural metabolic intermediate 5-(2-hydroxyethyl)-4-methylthiazole (1), and could therefore be a competitive inhibitor or substrate for the phosphokinase. If *in vivo* phosphorylation occurs then the possibility exists that 2-fluorothiamine phosphate and pyrophosphate might be formed.

Aryl fluorides are conveniently synthesised by the thermal decomposition of diazonium fluoroborates,[13] but for fluoroazoles,[14-17] photochemical decomposition is often a more satisfactory procedure. A preliminary study with 4,5-dimethylthiazole-2-diazonium hexafluorophosphate (the tetrafluoroborate salt [18] could not be isolated) showed that both thermal and photochemical decomposition gave 2-fluoro-4,5-dimethylthiazole in similar yield.

2-Amino-5-(2-hydroxyethyl)-4-methylthiazole [19,20]

gave on diazotisation water-soluble diazonium tetrafluoroborate and hexafluorophosphate salts, so that the protocol for thermal decomposition was not possible. Photochemical decomposition in aqueous solution however gave 2-fluoro-5-(2-hydroxyethyl)-4-methylthiazole (2).

The pK_a of 2-fluoro-5-(2-hydroxyethyl)-4-methylthiazole (2) was measured spectrophotometrically against the Hammett acidity function, H_0,[21-23] and was −1.44, some 5.26 units below that of 5-(2-hydroxyethyl)-4-methylthiazole (1) and comparable with the recently reported pK_a values of 2-chloro- and 2-bromo-thiazoles (−0.75 and −0.86 respectively [23]). The low pK_a was reflected in its inability to form a picrate salt, whereas 5-(2-hydroxyethyl)-4-methylthiazole (1) does.[24] Since it seems probable that the low pK_a will be paralleled by low nucleophilicity, 2-fluoro-5-(2-hydroxyethyl)-4-methylthiazole phosphate may not serve as a substrate for the formation of 2-fluorothiamine phosphate. It could however be a substrate or inhibitor of 5-(2-hydroxyethyl)-4-methylthiazole phosphokinase and indeed showed activity against *E. coli* ATCC 9637 at a concentration of 6 mM.

5-(2-Fluoroethyl)-4-methylthiazole (3) has also been prepared from the natural metabolic intermediate 5-(2-hydroxyethyl)-4-methylthiazole (1) by reaction with diethylaminosulphur trifluoride (DAST).[25] Moreover it was converted into the fluorothiamine (4) by reaction with 4-amino-5-bromomethyl-2-methylpyrimidine.[26,27] Both showed bacteriostatic activity against *E. coli* ATCC 9637 at concentrations of 6 and 4.4 mM respectively. 5-(2-Fluoroethyl)-4-methylthiazole is also being tested for pharmacological properties, since 5-(2-chloroethyl)-4-methylthiazole is a versatile pharmacological agent.[28]

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Preparative t.l.c. was performed on 20 cm × 20 cm silica gel H plates containing Fluor, bands being located with u.v. light at 254 or 366 nm. ^1H and ^{19}F N.m.r. spectra were recorded on a Perkin-Elmer R32 spectrometer. ^{19}F Chemical shifts are in p.p.m. from external trifluoroacetic acid, downfield resonances being assigned a negative value. High-resolution mass spectra were measured on a V.G. Micromass 7070F mass spectrometer. U.v. and turbidimetric measurements were performed on a Unicam SP 1800 spectrophotometer and i.r. spectra measured on a Unicam SP 1000 spectrophotometer. pH Measurements were made on a Radiometer type TTT1c pH meter. *E. coli* ATCC 9637 was obtained from the American Type Culture Collection, and inhibition of growth was tested as described by Iwashima and Nose.[6]

The following compounds were synthesised by literature methods: 2-amino-5-(2-hydroxyethyl)-4-methylthiazole,[19] 5-(2-hydroxyethyl)-4-methylthiazole,[20] 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide,[26,27] and diethylaminosulphur trifluoride (DAST).[25] Chloroform used for reactions involving DAST was freed from ethanol and distilled. 2-Amino-4,5-dimethylthiazole hydrobromide was obtained from Aldrich Chemical Co., hexafluorophosphoric acid (65% solution in water) from Cambrian Chemicals Ltd., and tetrafluoroboric acid (40% solution in water) from British Drug Houses Ltd.

2-Fluoro-5-(2-hydroxyethyl)-4-methylthiazole (2).—(a) Via *the diazonium fluoroborate*. 2-Amino-5-(2-hydroxyethyl)-4-methylthiazole (1.20 g, from 1.5 g, of its crystalline hydrochloride) was dissolved in fluoroboric acid solution (20 cm³) and cooled to −5 °C. Solid sodium nitrite (1.2 g) was added in portions over 20 min to the vigorously stirred solution. The cold green solution was transferred to a Pyrex tube and kept at −5 °C with an ice–salt mixture, whilst being photolysed with a 450-W medium-pressure mercury-vapour lamp (Hanovia). Gas evolution was complete after 4 h. The solution was brought to pH 8.0 with solid sodium hydrogencarbonate and then extracted with ether continuously for 6 h. The residue, after removal of the ether from the extract, was chromatographed on silica gel (35 g) with light petroleum (b.p. 40—60 °C)–ethyl acetate (1 : 3 v/v) to give 2-fluoro-5-(2-hydroxyethyl)-4-methylthiazole (2) as a pale yellow liquid (0.35 g, 28%), δ_H ($CDCl_3$) 3.77 (t, *J* 6.2 Hz, 2 H, CH_2CH_2OH), 3.35 (br s, 1 H, CH_2OH), 2.84 (dt, *J* 6.2 and 3.0 Hz, 2 H, CH_2CH_2OH), and 2.18 (s, 3 H, Me); δ_F ($CDCl_3$) + 6.00 (t, *J* 3.0 Hz) (Found: *m/e* 161.029 8. Calc. for C_6H_8FNOS: *M*, 161.031 0).

(b) Via *the diazonium hexafluorophosphate*. The same amounts of material were used as in (a) except that the fluoroboric acid solution was replaced by hexafluorophosphoric acid solution (7 cm³). The yield of 2-fluoro-5-(2-hydroxyethyl)-4-methylthiazole (2) was 0.30 g (24%).

2-Fluoro-4,5-dimethylthiazole.[18]— 2-Amino-4,5-dimethylthiazole (0.5 g, from its hydrobromide) was suspended in water (4 cm³) and 10M-HCl added (2 cm³) followed by sodium nitrite (0.5 g). Addition of hexafluorophosphoric acid (2 cm³) to the clear green solution gave immediately a yellow precipitate, which was filtered off, washed, and dried. The 4,5-dimethylthiazole-2-diazonium hexafluorophosphate (0.5 g, 44%), m.p. 105 °C (decomp.) had δ_H ([$^2H_6$]-DMSO) 2.0 (s, 2 Me); δ_F ([$^2H_6$]DMSO) −5.5 (d, J_{PF} 723 Hz); $\nu_{max.}$ (Nujol) 2 240 cm^{-1} ($-N_2^+$).

Pyrolysis. The dry diazonium salt was added in portions to a preheated (120 °C) round-bottomed flask. Extraction of the residue with ether and preparative t.l.c. using MeOH–$CHCl_3$ (1 : 9 v/v) as eluant gave 2-fluoro-4,5-dimethylthiazole (50 mg, 22%).

Photolysis. A suspension of the 4,5-dimethylthiazole-2-diazonium salt in water was photolysed by a 450-W medium-pressure mercury-vapour Hanovia lamp, a stream of nitrogen being used to agitate the suspension. After evolution of nitrogen ceased, the product was extracted and purified by preparative t.l.c. to give 2-fluoro-4,5-dimethylthiazole (57 mg, 25%) δ_H ($CDCl_3$) 2.00 and 2.16 (s, Me); δ_F ($CDCl_3$) + 6.0 (s).

5-(2-Fluoroethyl)-4-methylthiazole (3).—To a solution of DAST (1.45 g) in chloroform (5 cm³) at 0 °C was added dropwise a solution 5-(2-hydroxyethyl)-4-methylthiazole (1.0 g) in chloroform (5 cm³). After addition was complete the mixture was allowed to warm up to room temperature (30 min) and water (10 cm³) was added dropwise with vigorous stirring. The chloroform layer was washed with saturated sodium hydrogencarbonate solution, followed by saturated NaCl solution, dried ($MgSO_4$), and the solvent removed to give a pale yellow liquid which was chromatographed on silica gel (100 g), eluting with ethyl acetate. *5-(2-Fluoroethyl)-4-methylthiazole* (3) (0.55 g, 55%) had

δ_H ($CDCl_3$) 4.55 (dt, J_{HH} 6.0, J_{HF} 47.5 Hz, 2 H, FCH_2CH_2), 3.14 (dt, J_{HH} 6.0, J_{HF} 24 Hz, 2 H, FCH_2CH_2), 2.40 (s, 3 H, Me), and 8.57 (s, 1 H, N=CH); δ_F ($CDCl_3$) + 140 (tt, J_{HF} 24.0 and 47.5 Hz); and formed a picrate, m.p. 107—110 °C (Found: C, 38.5; H, 2.9; N, 14.8. $C_{12}H_{11}FN_4O_7S$ requires C, 38.5; H, 3.0; N, 15.0%), and a methanesulphonate salt, m.p. 90—92 °C.

3-[(4-*Amino*-2-*methylpyrimidin*-5-*yl*)*methyl*]-5-(2-*fluoroethyl*)-4-*methylthiazolium Bromide Hydrobromide* (4).—To a solution of 5-(2-fluoroethyl)-4-methylthiazole (3) (50 mg) in butan-1-ol (0.3 cm^3) was added 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide (63 mg) and the mixture heated at 100—120 °C for 15 min. The pyrimidine dissolved and shortly afterwards crystals appeared. Hot ethanol (1 cm^3) was added and the solution allowed to cool. The product was filtered off and recrystallised from ethanol to give the *thiazolium salt* (4) (22 mg, 23%), m.p. 253 °C (decomp.), δ_H (D_2O) 3.07 (s, 3 H, Me), 3.15 (s, 3 H, Me), 3.93 (dt, J_{HH} 6.0, J_{HF} 24.0 Hz, 2 H, FCH_2CH_2), 5.26 (dt, J_{HH} 6.0 and J_{HF} 47.5 Hz, 2 H, FCH_2CH_2), 6.08 (s, 2 H, CH_2), 8.54 (s, 1 H, N=CH), and 11.14 (s, 1 H, $\overset{+}{N}$=CHS, disappears in D_2O), δ_F (D_2O) + 142 (tt, J_{HF} 24.0 and 47.5 Hz) (Found: C, 32.0; H, 4.4; N, 11.7. $C_{12}H_{19}Br_2FNS{\cdot}H_2O$ requires C, 32.3; H, 4.3; N, 12.3%).

pK_a *Determinations.*—The pK_a values of the thiazoles (1) and (2) were measured spectrophotometrically.[21-23] To a stock solution of AnalaR sulphuric acid (2 cm^3) of known molarity in a quartz cuvette was added a solution of 2-fluorothiazole in water (100 μl, 1 mg cm^{-3}) containing potassium chloride (100 mM). After mixing, the absorbance was recorded against the appropriate blank solvent. The H_0 value of the solution was obtained by interpolation from the data of Paul and Long.[29] For the thiazole (1): free base $\lambda_{max.}$ 249 nm (ε 4 190), conjugate acid $\lambda_{max.}$ 258 nm (ε 4 450), $\lambda_{determination}$ 270 nm; for thiazole (2): free base $\lambda_{max.}$ 242 nm (ε 3 969), conjugate acid 257 nm (ε 3 220), $\lambda_{determination}$ 270 nm. The pK_a values were evaluated by least-squares regression analysis of plots of $\log_{10}$ [BH^+]/[B] against H_0 or pH.

The authors gratefully acknowledge the award of an S.R.C. studentship (to B. V. L. P.), and assistance with the antibacterial testing by Mr. J. W. Keeping.

[9/1797 *Received*, 12*th November*, 1979]

REFERENCES

1 G. M. Brown, *J. Biol. Chem.*, 1962, **237**, 536.
2 A. D. Barton and L. L. Rogers, in ' The Biochemistry of the B Vitamins,' eds. R. J. Williams, R. E. Eakin, G. Beerstecher, jun., and W. Shive, Reinhold, New York, 1950, p. 684.
3 T. L. V. Ulbricht and C. C. Price, *J. Org. Chem.*, 1956, **21**, 567.
4 H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, 1962, **27**, 3614.
5 J. A. Gutowski and G. E. Lienhard, *J. Biol. Chem.*, 1976, **251**, 2863.
6 A. Iwashima and Y. Nose, *J. Biochem.* (*Japan*), 1967, **62**, 537.
7 Y. Nose and A. Iwashima in ' Current Aspects of Biochemical Energetics,' eds. N. O. Kaplan and E. P. Kennedy, Academic Press, New York, 1966, p. 343.
8 G. M. Brown in ' The Vitamins,' ed. W. H. Sebrell, jun., and R. S. Harris, Academic Press, New York, vol. 5, 1972, p. 122.
9 D. E. Metzler, ' Biochemistry,' Academic Press, New York, 1977, p. 438.
10 R. Breslow, *J. Amer. Chem. Soc.*, 1957, **79**, 1762.
11 M. Schlosser, *Tetrahedron*, 1978, **34**, 3.
12 Ciba Foundation Symposium on Carbon Fluorine Compounds, ' Chemistry, Biochemistry and Biological Activities 1972,' Elsevier, Amsterdam.
13 A. Roe, *Org. Reactions*, 1949, **5**, 193.
14 K. L. Kirk and L. A. Cohen, *J. Amer. Chem. Soc.*, 1973, **95**, 4619.
15 K. L. Kirk, W. Nagai, and L. A. Cohen, *J. Amer. Chem. Soc.*, 1973, **95**, 8389.
16 K. L. Kirk and L. A. Cohen, *J. Org. Chem.*, 1973, **38**, 3647.
17 F. Fabra, C. Gálvez, A. Gonsalez, P. Viladoms, and J. Vilarrasa, *J. Heterocyclic Chem.*, 1978, **15**, 1227.
18 C. Grünert, H. Schellong, and K. Wiechert, *Z. Chem.*, 1970, **10**, 116.
19 A. R. Todd, F. Bergel, H. L. Fraenkel-Conrat, and A. Jacob, *J. Chem. Soc.*, 1936, 1601.
20 E. R. Buchman, *J. Amer. Chem. Soc.*, 1936, **58**, 1803.
21 C. D. Johnson, A. R. Katritzky, B. J. Ridgewell, N. Shakir, and A. M. White, *Tetrahedron*, 1965, **21**, 1055.
22 A. R. Katritzky, C. Ogretir, H. O. Tarhan, H. M. Dou, and J. Metzger, *J.C.S. Perkin II*, 1975, 1614.
23 L. Forlani and P. De Maria, *J.C.S. Perkin II*, 1979, 163.
24 P. G. Linnet and J. Walker, *J. Chem. Soc.* (*C*), 1967, 796.
25 W. J. Middleton, *J. Org. Chem.*, 1975, **40**, 574.
26 R. Grewe, *Z. physiol. Chem.*, 1936, **242**, 89.
27 H. Andersag and K. Westphal, *Ber.*, 1937, **70**, 2035.
28 *Acta Psychiatrica Scandinavica*, Suppl., 1966, **192**, 45.
29 M. A. Paul and F. A. Long, *Chem. Rev.*, 1957, **57**, 1.