6-Fluoropyridoxol

6-FPOL

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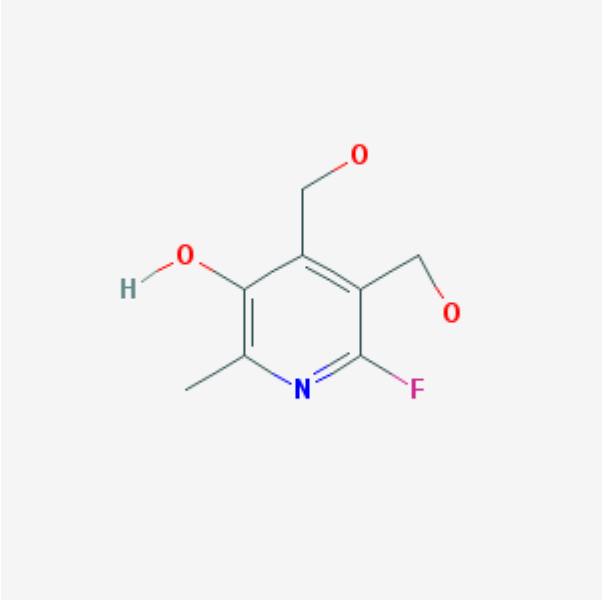
Chemical name:	6-Fluoropyridoxol
Abbreviated name:	6-FPOL
Synonym:	2-Fluoro-5-hydroxy-6-methyl-3,4-pyridinedimethanol, 2-fluoro-3,4-di(hydroxymethyl)-5-hydroxy-6-methyl-pyridine
Agent Category:	Compound
Target:	Proton (H^+)
Target Category:	Protonation and deprotonation of the 3-phenolic OH in different pH environments that lead to chemical shift changes of ^{19}F at the 6 position.



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Method of detection:	^{19}F-Magnetic Resonance Spectroscopy (MRS)	
Source of signal/ contrast:	^{19}F	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on the above structure for additional information in PubChem.

Background

[PubMed]

6-Fluoropyridoxol (6-FPOL) is a fluorinated vitamin B_6 derivative developed for measuring transmembrane pH gradient *in vivo* with ^{19}F magnetic resonance spectroscopy (^{19}F-MRS) (1, 2).

Magnetic resonance imaging and MRS imaging are evolving nuclear magnetic resonance (NMR) techniques that can be used for non-invasive evaluation of anatomic and metabolic features of pathologies (3, 4). MRS can detect metabolites that contain proton, phosphorus, fluorine, or other nuclei. Both ^{1}H- and ^{31}P-MRS have revealed significant disturbances in amino acids, lipids, and phosphorus-containing metabolites *in vivo* (5, 6). Cellular pH has been found to play a regulatory role in most cellular processes, from the activity of individual enzymes to coordinated development of an organism (7). Many metabolic enzymes exhibit optimal activity in a narrow pH range. There are also significant pH gradients within and between cellular compartments. Compared with normal tissue, tumor tissue has a reversed pH gradient with an acidic extracellular compartment and a basic intracellular compartment. Mechanical activity of the heart is greatly influenced by pH, and myocardial ischemia can lead to hypoxia and acidosis. Clinically, MRS can be used to monitor cellular pH and detect pH changes in response to therapy. Theoretically, MRS chemical shift imaging may allow *in vivo* mapping of tissue/ organ pH changes.

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Many molecules exhibit chemical shift response to pH changes, and measurements of these chemical shifts are directly related to pH (7). *In vivo* MRS pH measurement can be performed with endogenous molecules such as inorganic phosphate in ^{31}P-MRS. However, pH measurement in whole tissues by use of endogenous molecules is not always successful because of crowded spectra, broad lines, or low molecule concentrations. Exogenous molecules have been found to be useful as reporter probes to facilitate *in vivo* pH measurement. Among these molecules, ^{19}F-MRS chemical shift molecules have distinct advantages of a large chemical shift range, high gyromagnetic ratio, high detection sensitivity, and no background signal from tissues. Mason (7) studied various fluorinated vitamin B_6 analogs as ^{19}F-MRS agents to measure intracellular pH (pH_i) and extracellular pH (pH_e). Mehta et al. (2) evaluated one of these analogs, 6-FPOL, and found that the molecule exhibited exceptional sensitivity to changes in pH and could be used to measure pH_i and pH_e simultaneously. The response of ^{19}F-MRS chemical shifts to pH in the molecule is considered to be caused primarily by protonation and deprotonation of the 3-phenolic OH. This affects the electronic environment around fluorine at position 6, which is very pronounced because of the *para* location.

Synthesis

[PubMed]

Korytnyk and Srivastava (8) first reported the synthesis of 6-halogen-substituted vitamin B_6 analogs. Mason (7) described it in three steps starting from pyridoxine hydrochloride. Diazotization of pyridoxine with benzene diazonium chloride gave 6-phenazopyridoxol. Dithionite reduction or catalytic reduction (Pd/C) produced 6-aminopyridoxol. 6-FPOL was then produced from 6-aminopyridoxol by a modified Schieman reaction. Specifically, 6-aminopyridoxol in 40% HBF_4 was reacted with $NaNO_2$ at 10 °C for 2 h. After addition of 5 N NaOH at 10 °C to pH 3-4, the solution was extracted with diethyl ether, washed with water, dried, and concentrated. The crystalline material was recrystallized from diethyl ether. The final yield was 34%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Mehta et al. (2) used 7-Tesla (282.3 MHz) NMR to study 6-FPOL in fresh whole blood obtained from rabbits. 6-FPOL exhibited a single sharp ^{19}F-NMR resonance in aqueous solution or plasma with a line width of ~20 Hz. The ^{19}F-NMR chemical shift of 6-FPOL was very sensitive to pH with a change of 9.76 ppm between acid and base. Coefficients of the Henderson-Hasselbach equation were determined as p*K*a = 8.2, δ (acid) = –9.85 ppm, and δ (base) = –19.61 ppm. 6-FPOL produced two distinct, well-resolved ^{19}F-NMR resonances at –10.72 ± 0.03 ppm (intracellular compartment) and –11.34 ± 0.06 ppm (extracellular compartment), which corresponded to pH 7.19 ± 0.02 and 7.45 ± 0.02, respectively. The molecule had a chemical shift range of ~10 ppm. The pH-dependent ^{19}F-NMR chemical shift of 6-FPOL was independent of temperature and the presence of

metal ions or plasma proteins. 6-FPOL penetrated and left the red blood cells rapidly, but exchange was sufficiently slow to provide separate resonances for intra- and extracellular compartments. Acid-base exchange was in the fast-exchange regime so that a single narrow line was observed for each compartment. In a separate experiment using blood saturated with carbon monoxide to reduce intracellular paramagnetic line broadening from deoxyhemoglobin, ^{31}P-NMR indicated an intracellular pH ~7.14 ± 0.06.

Hunjan et al. (9) evaluated 6-FPOL in perfused rat hearts, using a 9.4-Tesla NMR (376 MHz) spectrometer. The addition of 6-FPOL to a single heart showed two well-resolved peaks at –10.63 ± 0.01 ppm (intracellular compartment; n = 3 repeated measurements) and –11.53 ± 0.04 ppm (extracellular compartment), corresponding to pH_i = 7.14 ± 0.01 and pH_e = 7.52 ± 0.02, respectively. In eight hearts, the measurements were similar, in the range of –10.43 to –10.69 ppm (intracellular compartment) and –11.50 to –12.45 ppm (extracellular compartment), corresponding to mean pH_i of 7.11 ± 0.07 and pH_e of 7.60 ± 0.08, respectively. The ^{31}P-NMR chemical shift of inorganic phosphate gave similar measurements for pH_i (7.11 ± 0.04; n = 7), and external electrode measurement gave a pH_e of 7.63 ± 0.33 (n = 5). On addition of 6-PFOL (3.6 mM), hearts occasionally showed an immediate but transient 10-40% reduction in developed pressure, lasting 3-6 min, followed by complete recovery. 6-FPOL ^{19}F-MRS showed the changes in pH_i and pH_e when artificial metabolic alkalosis or acidosis was induced by sodium hydroxide or hydrochloric acid. In both instances, pH_e changed significantly but pH_i remained unchanged. In respiratory alkalosis induced by reducing the CO_2 concentration from 5% to 0%, both pH_i and pH_e changed simultaneously. Total global ischemia induced by halting the flow of perfusate caused the intracellular and extracellular peaks to merge together as an indication of acidosis.

He et al. (1) studied 6-FPOl and a series of fluorinated vitamin B_6 analogs in whole blood and perfused rat hearts, using a 7-Tesla (282 MHz) NMR spectrometer. They found that the pyridine core of vitamin B_6 was readily amenable to many chemical modifications. The modification of the 4- and 5- position hydroxymethyl moieties produced modification of the p*K*a with relatively minor changes in chemical shift and chemical shift range. The introduction of an aminomethyl group at these positions appeared to cause the p*K*a to shift toward the acidic range. The introduction of the electron-withdrawing carboxyl group caused a shift toward base.

Korytnyk and Srivastava (8) found that 6-FPOL appeared to inhibit *in vitro* growth of tumor cells with an inhibitory concentration (ID_{50}) of ~0.1 mM for adenocarcinoma (TA-3) cells and 25 μM for sarcoma (S-180) cells.

Animal Studies

Rodents

[PubMed]

Korytnyk and Srivastava (8) injected a single i.p. dose of 6-FPOL in mice and found that the mice survived a dose of 100 mg/kg

Mason (7) reported that transmembrane pH gradient of tumors could be measured by ^{19}F-MRS in rats bearing Dunning AT1 prostate rat tumors after i.p administration of 6-FPOL (200 mg/rat; ~800 mg/kg) with no obvious acute toxicity.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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