with other studies of the metabolism of pyridoxine in which this analog was tested.[5,6] However, replacement of the 2-methyl group with the bulkier isopropyl group (II) or the addition of a chloro substituent at position 6 (IV) resulted in analogs with only one-fourth the activity of pyridoxine.

Experimental Section[7]

Synthesis. 2-Isopropyl-3-hydroxy-4,5-bis(hydroxymethyl)-pyridine (II).—The synthesis of this analog is illustrative of the method used for the other compounds. 3-Cyano-4-ethoxycarbonyl-6-isopropyl-2-pyridone was prepared by dissolving the sodium salt of ethyl isobutyropyruvate[8] (36 g, 0.174 mole) and cyanoacetamide (15 g, 0.18 mole) in 250 ml of absolute ethanol and refluxing for 2 hr. After cooling to room temperature, 35 ml of concentrated HCl made up to 210 ml with ice and water was slowly added with stirring. The product, after filtration and washing with ice water, amounted to 29 g (71%) and melted at 191–193°.

Anal. Calcd for $C_{12}H_{14}N_2O_4$: C, 61.5; H, 5.6; N, 12.0. Found: C, 61.6; H, 5.6; N, 11.9.

This compound (137.5 g, 0.585 mole) was then nitrated with fuming HNO_3–Ac_2O as described by Wuest, *et al.*,[2] to give 121.6 g (74%) of 3-cyano-4-ethoxycarboxyl-5-nitro-6-isopropyl-2-pyridone melting at 233–234°.

Anal. Calcd for $C_{12}H_{13}N_3O_6$: C, 51.6; H, 4.7; N, 15.0. Found: C, 52.1; H, 4.8; N, 14.7.

The chlorinated compound, 2-isopropyl-3-nitro-4-ethoxycarbonyl-5-cyano-6-chloropyridine, was prepared by heating together the nitropyridone (123 g, 0.44 mole) and PCl_5 (104 g, 0.5 mole) at 120–130° for 2 hr. The liquid reaction mixture was cooled and the $POCl_3$ was removed *in vacuo*. The viscous residue was added to crushed ice and stirred until solidification was complete. Recrystallization from ethanol gave 97 g (74%) of product melting at 75–77°.

Anal. Calcd for $C_{12}H_{12}ClN_3O_4$: C, 48.4; H, 4.1; N, 14.1. Found: C, 48.9; H, 4.3; N, 13.5.

Reduction of the 3-nitro group to an amino group with iron powder and HCl[2] gave 2-isopropyl-3-amino-4-ethoxycarbonyl-5-cyano-6-chloropyridine (mp 167–170°).

Anal. Calcd for $C_{12}H_{14}ClN_3O_2$: C, 54.83; H, 5.27; N, 15.7. Found: C, 54.39; H, 5.53; N, 15.92.

Removal of the 6-chloro group by hydrogenolysis followed by acid hydrolysis of the ester and cyano groups as described by Wuest, *et al.*,[2] gave the dicarboxylic acid compound, 2-isopropyl-3-aminopyridine-4,5-dicarboxylic acid melting at 218°. Diazotization gave 2-isopropyl-3-hydroxypyridine-4,5-dicarboxylic acid (mp 230–233°). Reduction of this compound (1.35 g, 5.56 mmoles) with $NaBH_4$–$AlCl_3$ in diglyme according to Blackwood, *et al.*,[3] gave 0.6 g (46%) of II, mp 193–195° (lit.[9] mp 192°).

2-Methyl-3-amino-4,5-bis(hydroxymethyl)-6-chloropyridine (III).—The synthesis of this analog up to the aminochloro compound, 2-methyl-3-amino-4-ethoxycarbonyl-5-cyano-6-chloropyridine, was essentially as described previously.[2] Alkaline hydrolysis,[10] however, was used to obtain the dicarboxylic acid, 2-methyl-3-amino-6-chloropyridine-4,5-dicarboxylic acid. Reduction with $NaBH_4$–$AlCl_3$ in diglyme gave III, mp 217–219° (lit.[10] mp 220–222°).

2-Methyl-3-hydroxy-4,5-bis(hydroxymethyl)-6-chloropyridine (IV).—Analog III was diazotized by treating a 1-g solution in 2 *N* H_2SO_4 with 1 g of $NaNO_2$ in 5 ml of water. The temperature was maintained at 70–80° for 15 min. After cooling and neutralizing to pH 7 with NaOH, the solution was evaporated to dryness *in vacuo*. The residue was extracted with 50 ml of hot absolute ethanol and filtered. The filtrate was taken to dryness and the residue remaining was extracted with ether in a Soxhlet extractor. Reducing the volume of the ether extract to 5 ml followed by standing at room temperature for 2–3 hr resulted in the formation of crystals, which, after filtering and drying, amounted to 0.175 g (21%), mp 188–192° (lit.[3] mp 192–193°).

2-Methyl-4,5-bis(hydroxymethyl)pyridine (V). 3-Cyano-4-ethoxycarbonyl-6-methyl-2-pyridine[2] was chlorinated to give 2-chloro-3-cyano-4-ethoxycarbonyl-6-methylpyridine and then hydrogenated using Pd–$BaCO_3$ as previously described[10] to give 2-methyl-4-ethoxycarbonyl-5-cyanopyridine (mp 57–58°, lit.[9] mp 58°). Alkaline saponification of this compound with 15% NaOH for 16 hr at 120° gave, after acidification of the salt with 25% HCl, 2-methylpyridine-4,5-dicarboxylic acid (mp 249–250°, lit.[12] mp 249–251°). Reduction of this diacid with $NaBH_4$–$AlCl_3$ in diglyme gave V, mp 211–212° (lit.[13] mp 202–203°).

2-Methyl-3-amino-4,5-bis(hydroxymethyl)pyridine (VI). The procedure employed for the 2-isopropyl analog (II) was followed, using ethyl acetopyruvate in the initial condensation step in place of ethyl isobutyropyruvate. The final step, reduction of the 4,5-dicarboxylic acid with $NaBH_4$–$AlCl_3$, gave the expected product, mp 192–193° (lit.[3] mp 197°).

Enzymatic Assay Procedure. The coenzyme was partially purified from baker's yeast essentially as described by Morino and Sakamoto.[4] The assay mixture contained, in a final volume of 2.5 ml, 2.5 μmoles of NADPH, 100 μmoles of Na_2CO_3–$NaHCO_3$ buffer (pH 9.0), and the appropriate amount of enzyme and substrate. The substrate concentration in each case was the amount required to give maximum activity. The substrate solution was added after the other components had incubated at 37° for 2 min. The reaction was stopped after 10 min by adding 0.3 ml of 100% (w/v) trichloroacetic acid. The precipitated protein was removed by filtration. Aliquots of the filtrate were added to 0.2 ml of phenylhydrazine solution[5] and the volume was adjusted to 4 ml. After heating at 60° for 20 min to develop the color of the phenylhydrazone, the absorbance at 410 mμ was measured in a Bausch and Lomb Spectronic 20 colorimeter.

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Metabolism of 5-(3-Pyridyl)tetrazole

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In a recent publication Holland and Pereira described the synthesis and lipolysis inhibitory property of a group of heterocyclic tetrazoles.[1] One member of this group 5-(3-pyridyl)tetrazole (**1**) was shown to be considerably less active than nicotinic acid in an *in vitro* assay, but was equipotent to nicotinic acid when tested *in vivo* in the dog.[1] More striking, however, was the difference in the duration of pharmacological effect between **1** and nicotinic acid. In the dog nicotinic acid produced a fall in plasma free fatty acid which lasted approximately 1 hr, whereas the duration of decreased plasma free fatty acid caused by **1** lasted approximately 5 hr. Since nicotinic acid is known to be extensively metabolized, it was speculated that the improved duration of action of **1** over nicotinic acid may be attributable to greater metabolic stability of **1**. Results reported here indeed show that 5-(3-pyridyl)-

(1) G. F. Holland and J. N. Pereira, *J. Med. Chem.*, **10**, 149 (1967).

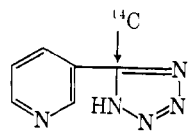
tetrazole (**1**) resists metabolic degradation by rat, dog, and man.

When ^{14}C-labeled **1** was administered intravenously to rats approximately 65% of the radioactivity was recovered in urine and approximately 20% in feces within the first 24 hr (Table I). Subsequent excreta

TABLE I
ADMINISTRATION OF ^{14}C-5-(3-PYRIDYL)TETRAZOLE TO THE RAT AND DOG

Species	Dose, mg/kg	% of admin radioactivity recd, 0–24 hr after dose: Urine	Feces
Rat #1	60 iv	74	18
#2	200 iv	60	24
Dog #1	50 oral (gelatin capsule)	68	
#2	50 iv	87	

contained a small or insignificant quantity of radioactivity. Papergram chromatographic assays of urine or fecal extracts followed by radioactive strip scanning showed only one single radioactive peak with R_f identical with the control 5-(3-pyridyl)tetrazole (**1**). Similar



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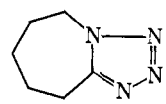

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results were obtained following the oral and intravenous administration of **1** to dogs. To further establish the identity of recovered radioactivity a portion of dog urine was subjected to ion-exchange chromatography. The eluted radioactivity was diluted with unlabeled **1** and recrystallized to constant specific activity. Paper chromatography of the recrystallized material indicated homogeneity of label and mass.

In human studies the administration of unlabeled **1** in doses ranging from 8 to 25 mg/kg, resulted in the 24-hr urinary recovery of 30–95% of the administered dose as unchanged **1**.[2] The observed metabolic stability of **1** appears to be unique among 3-substituted pyridines. Following the administration of ^{14}C-3-acetylpyridine to rats, 25% of the dose is recovered in urine as 3-acetyl-6-pyridone and 0.3% as N-methyl-3-acetyl-6-pyridone. With a dose as high as 1000 mg/kg, no unchanged 3-acetylpyridine could be found in rat urine.[3] The major portion of ^{14}C-labeled nicotinic acid administered to the rat and dog is recovered methylated as the N-methyl quaternary compound, 1-methylnicotinamide.[4] Thus, in the example of 3-substituted pyridines, the presence of a tetrazole substituent prevents metabolic alterations of the pyridine moiety.

1,5-Pentamethylenetetrazole (**2**) is another tetrazole derivative known to be metabolically stable. Approximately 75% of the dose of **2** is recovered in the urine as unchanged drug after administration to rats.[5] Although the heterocyclic portion of **2** offers no convenient "handle" for conversion, the saturated ring would appear to be a suitable substrate for oxidation. Approximately 30% of cyclohexane administered to rabbits is recovered unchanged, but the major portion, approximately 60%, is metabolized *via* hydroxylation.[6] Williams[5] discusses the possibility that **2** is excreted as a labile conjugate but reverts to **2** under the acid conditions of urine. Following the administration of **1** to rats or dogs no attempt was made to alter the pH of urine, and aliquots of urine were subjected to paper chromatography shortly after collection. All papergrams after scanning showed a single peak of radioactivity with a mobility consistent with **1**; no evidence for the presence of a radioactive conjugate was detected.

Until additional data on the metabolism of tetrazoles become available, it will not be possible to generalize from the two examples discussed that the presence of a tetrazole group in a drug confers characteristics rendering the entire molecule unsuitable for metabolic transformations.

Experimental Section

Synthesis of ^{14}C-Labeled 5-(3-Pyridyl)tetrazole (1). Nicotinonitrile-7-^{14}C.—Nicotinonitrile was synthesized from nicotinamide by the method of Teague and Short.[7] A mixture of nicotinamide-7-^{14}C, 21.2 mg (2 mcuries, New England Nuclear Corp.), and 1.198 g of unlabeled nicotinamide was used in the synthesis; yield 509 mg (49% of 3-cyanopyridine).

5-(3-Pyridyl)tetrazole (1).—To 509 mg of ^{14}C-labeled 3-cyanopyridine was added 198 mg of unlabeled 3-cyanopyridine, 592 mg of NaN_3, 487 mg of NH_4Cl, 11.5 mg of LiCl, and 9.2 ml of dry DMF. The reaction mixture was stirred for 16 hr at 123–125°, allowed to cool, and the precipitate was recovered by centrifugation. The crude product was recrystallized once from 30 ml of hot water to yield 465 mg (46%) of **1**, specific activity 1.15 μcuries/mg, mp 235–236° dec, lit.[1] 238° dec.

Charles River CD rats weighing approximately 180 g and mongrel dogs, approximately 10 kg, were used. The drug was administered intravenously as an aqueous solution in the tail vein of rats or in the femoral artery of dogs.

Radioactivity in feces was recovered by EtOAc extraction of a water–fecal slurry acidified with 3 *N* HCl to pH 3.4. Urine radioactivity was recovered by chromatography over a column of Amberlite IR-120 resin which had been previously slurried in 1 *N* HCl and washed (H_2O) until neutral. Essentially all of the radioactivity applied to the column was recovered by elution with 1 *N* NH_4OH. The fractions containing radioactivity were combined and lyophilized, cold 5-(3-pyridyl)tetrazole was added, and the mixture was recrystallized to constant specific activity. When subjected to paper chromatography this material showed a single radioactive peak with R_f of **1**.

Paper chromatography was carried out in BuOH–dioxane–NH_4OH or BuOH–AcOH–H_2O. Papergrams were examined for radioactivity in an Atomic Accessories, Model RSC-180 continuous papergram strip scanner. All radioactive samples were measured in a Nuclear Chicago Model 725 liquid scintillation spectrometer. Quenching corrections were made by the internal standard procedure employing ^{14}C-toluene. All samples were assayed in a scintillator consisting of 0.3% 2,5-diphenyloxazole (PPO) and 0.01% *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in a mixture of 30% absolute ethanol and 70% toluene.

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(2) These experiments were conducted in collaboration with Drs. S. Gilgore and S. DeFelice of our Clinical Pharmacology Department.

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